Identification of potential drug target from *Plasmodium falciparum* and their novel inhibitors through molecular docking approach.

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(2014- 2016)

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"In the name of ALLAH The Most Gracious and The Most Beneficial"

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FINAL APPROVAL

It is certified that we have read the final project report submitted by **Mr. Ata Ullah** and it is our judgment that this project is of sufficient standard to warrant its acceptance by the International Islamic University, Islamabad for the MS Degree in Biotechnology.

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This humble effort is

Dedicated

To

My beloved

Parents

&

Teachers

Who inspired me for higher ideals of Life.

DECLARATION

I hereby solemnly declare that the work **"Identification of potential drug target from *Plasmodium falciparum* and their novel inhibitors through molecular docking approach"** presented in the following thesis is my own effort, except where otherwise acknowledged and that the thesis is my own composition. No part of the thesis has been previously presented for any other degree.

Dated: 04/08/16

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LIST OF ABBREVIATIONS

E. coli	*Escherichia coli*
P. falciparum	*Plasmodium falciparum*
P. vivax	*Plasmodium vivax*
P. malariae	*Plasmodium malariae*
P. ovale	*Plasmodium ovale*
P. knowlesi	*Plasmodium knowlesi*
A. darling	*Anopheles darling*
A. albitarsis	*Anopheles albitarsis*
A. nuneztovari	*Anopheles nuneztovari*
RBC	Red Blood Cell
PCR	Polymerase Chain Reaction
WHO	World Health Organization
CQ	Chloroquine
IPP	isopentenyl pyrophosphate
DMAPP	dimethylallyl pyrophosphate
FAS	Fatty acids biosynthesis pathway
MEP	methylerythritol 4-phosphate
FabZ	β-hydroxyacyl-ACP dehydratase
M. tuberculosis	*Mycobacterium tuberculosis*

E. coli	*Escherichia coli*
PABA	p-aminobenzoic acid
PDB	Protein Data Bank
wwPDB	Worldwide Protein Data Bank
MOE	Molecular Operating Environment
WSA	Weighted Surface Area
PPIs	protein–protein interactions
VS	Virtual screening
QSAR	Quantitative structure–activity relationship
LB	Luria Bertani
Ala	Alanine
Arg	Arginine
Asp	Aspartic acid
Cys	Cysteine
Glu	Glutamic acid
Gln	Glutamine
Gly	Glycine
His	Histidine
Leu	Leucine
Met	Methine

Phe	Phenylalanine
Pro	Proline
Ser	Serine

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ABSTRACT

Antimicrobial drug resistance has an impact on global health problem. The resistance against anti-malarial drug in the context of *Plasmodium falciparum* has gained much attention to discover and developed new anti-malarial compounds. B-hydroxyacyl-ACP dehydratase (FabZ) from *Plasmodium falciparum* has been reported as the drug target for antimalarial drug fosmidomycin. FabZ is an enzyme in the fatty acid biosynthesis pathway (FAS-II). It is essential for many pathogens and is absent in human, which makes it a good target for drug discovery. In order to find more lead chemicals for the drugs against this enzyme, we have performed *in silico* screening of novel antimalarial compounds with it structure. The structure of FabZ was downloaded from PDB database (PDB ID: 3AZ9) and validated by computational tools. Screening was done with the software package MOE and the ChemBridge database. Among the chemicals in the database, 35 molecules have been found to have good affinity to FabZ. Six selected molecules of them have an affinity score between -15.851/Mol and -9.260.36 KJ/Mol. To investigate the interaction pattern between these chemical and the target protein, these both were docked into the active site of FabZ. In the docked model, hydrogen bond interactions and hydrophobic interactions were found between the ligand and protein. Ligplot was used to demonstrate the details of the interactions. Conserved residues His133, Phe171, Met140 Gln145 and His133, involved in hydrogen bond interaction. Neutral nonpolar amino acids His98, Phe134, Pro141, Gly142, Val143, Glu147, Ala150, Gln151, Phe169, Leu170, Phe171 and Phe226, involved in hydrophobic interactions. To investigate is the chosen chemicals form stable complexs with the FabZ, we performed docking and molecular dynamics simulation with the complex structures. The results indicate the ligand/protein complexes stay stable over the course of the simulation, which is a good indication that the chosen chemicals have good potential to be developed as inhibitors against FabZ.

CHAPTER NO: 1

INTRODUCTION

1. Introduction

Malaria, a hematoprotozoan parasitic infection, greatly affects public health and economy worldwide. Both humans and animals suffer from this life-threatening disease. Five *Plasmodium* species, *Plasmodium vivax, Plasmodium falciparum, Plasmodium ovale and Plasmodium malariae* can cause malarial infection (Hayton *et al.*, 2007). *P. vivax and P. falciparum* can cause severe health problem in humans if not treated promptly (Hunter, 2007). Although the majority of deaths are caused by *P. falciparum*, recent study has shown that the *P. vivax* is associated with potentially life-threatening conditions (Xue *et al.*, 2013) and is more common outside Africa. Currently, 25% of malarial cases in Europe are caused by non-*falciparum* species (Aloy & Russell 2004). Although human infections caused by several *Plasmodium* species transmitted from higher apes have been documented, these species are mostly of limited public health importance (Jakalian *et al.*, 2002), except for *Plasmodium knowlesi*, a zoonotic species that causes malaria in macaques (Eisenreich *et al.*, 2004).

Malaria is one of the most threatening diseases with about 300 million cases every year in developing countries (Franceschini *et al.*, 2013). The death rate caused by *P. falciparum* is high because other species cause a milder form of malaria. Before 1949, approximately 30 million malaria cases occurred each year in China, with parasite incidence rates as high as 80,000/100,000 (Wallace *et al.*, 1995).

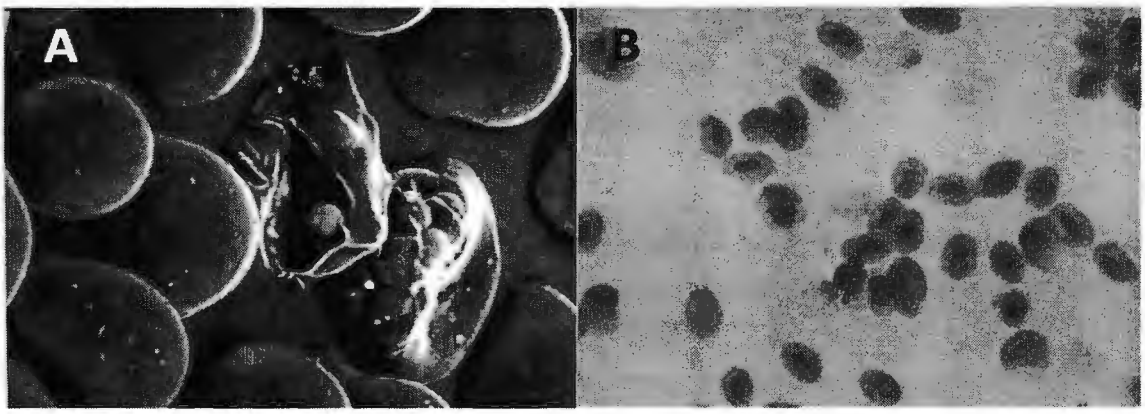


Figure 1.1 Blood stage parasites are responsible for the clinical manifestations of the disease (https://eukaryography.wordpress.com/category/microbes-2/)

Malarial infection is transmitted through the bite of female *Anopheles* mosquitoes carrying *Plasmodium* and by coming into contact with infected blood (Figure 1.1). To support egg development, mosquito probes the host environment for blood vessels. During blood feeding, the mosquito injects salivary gland proteins to inhibit blood coagulation. The parasites deposited into the skin can cross the surrounding cells and enter the circulation, thereby resulting in infection in liver cells. The maturation and reproduction stage occur in the liver, and the liver parasite load is similar between parasites injected intradermaly or intravenously (Rourke *et al.*, 2014).

1.1 Life cycle

There are three different phases of the lifecycle of *Plasmodium* and each phase is further divided into different stages (Figure 1.3).

In the liver phase (A), sporozoites, (infectious stage) - Infected *Anopheles* mosquito bite a healthy person and introduce the sporozoites to their blood stream, they are then migrated into the liver. Schizont developed from each sporozoite inside the liver. In case of *P. vivax* and *P. ovale,* the sporozoites developed into the hypnozoites (Eisenreich, 2004). Hypnozoites, the inactive form of sporozoite, is responsible for the backsliding of the disease a few months later of the infection that had occurred initially.

After the liver phase comes a phase called as the blood phase (B), several merozoites are released into the blood stream by the rupturing of schizont tissue (Hunter, 2004). After that RBCs are invaded by the merozoites. Within RBCs, trophozoites (Jakalian *et al.*, 2002), are developed by each merozoite and later transform to blood schizonts The blood schizonts multiply asexually, producing 16 to 32 merozoites The release of merozoites in the bloodstream occurs when the rupturing of RBCs takes place, allowing the invasion of more RBCs and continuous its growth through asexual reproduction. At this stage, clear clinical symptoms such as fever and chills appeared. Some merozoites also develop into gametocytes (Labute, 2008).

Blood phase is followed by the mosquito phase (C). During feeding on an infected person, gametocytes are ingested by the mosquito with blood (Figure 1.2 B). Thousands of

sporozoites are produced through asexual reproduction when gametocytes undergo asexual reproduction inside the midgut of mosquito, injection of these sporozoites into humans take place during a blood meal as soon as they are migrated to the mosquito's salivary glands.

The life cycle of *Plasmodium* starts with vector infection and is considered a keystone stage. *Anopheles darlingi*, among the 33 different species of *Anopheles* mosquito, is considered the only vector of malaria (Figure 1.2 A). Based on the anthropophilic behavior and population density, other species are categorized as secondary vectors of malaria (Sinka, *et al.*, 2012). Other *Anopheles* species, such as *A. albitarsis*, *A. nuneztovari*, and *A. triannulatus*, are infected by *P. falciparum* and *P. vivax*, but their role has not yet been elucidated as malarial vectors (Póvoa *et al.*, 2003). *P. vivax* infected the *A. aquasalis* in the Atlantic coast because of the tolerance of this mosquito to salt water (Póvoa *et al.*, 2003).

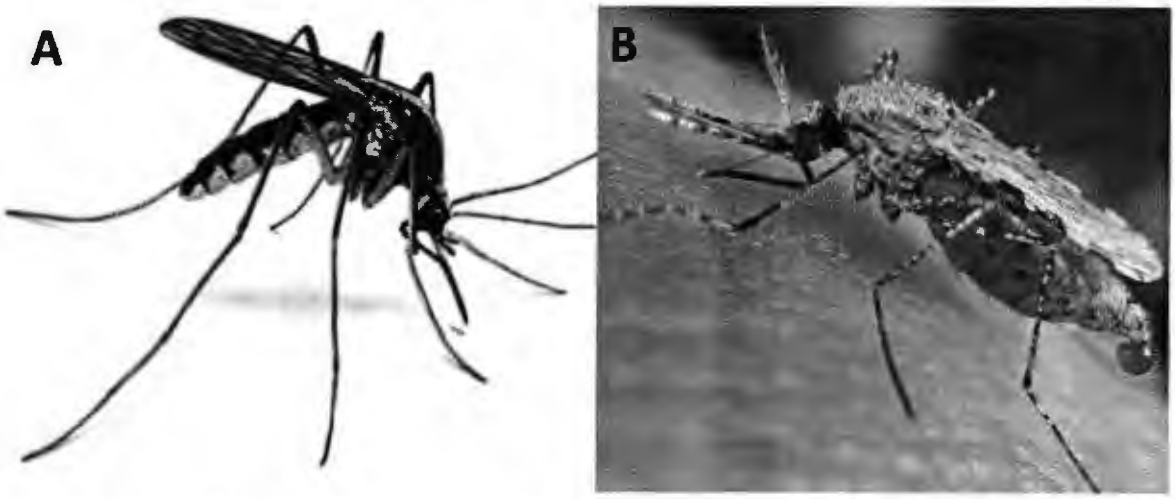


Figure 1.2 (A) The Anopheles darling, the main vector of malaria (B) The Anopheles mosquito during the blood meal
(http://web.stanford.edu/class/humbio153/MalariaVac/index.html)

The life cycle of mosquito starts when it ingests gametocytes from the infected host during a biting. Motile ookinetes, which are female gametocytes, are produced inside the alimentary canal of the mosquito by the fusion of male and female gametocytes that cross the midgut epithelium of mosquito to form oocysts (Shahabuddin and Kaslow 1994). The formation of oocytes in the midgut of mosquito indicates the infection. The number of oocytes indicates the rate of infection of a mosquito (Gamage-Mendis *et al.*, 1993).

According to the geographic range of *Anopheles* (Figure 1.2), the infection rate is considered an important parameter defining vector competence and thus considered a key indicator in the description of malaria dynamics and transmission biology. However, not all mosquitos show the ookinete stage of gametocytes that they ingested (Sinden *et al.*, 2004). Oocyte formation, which is the fusion of gametocytes, depends on many factors, such as mosquito immune factors, antimalarial drugs, human, gender ratio, and maturity and gametocyte density (Klein *et al.*, 1992). During sexual development in mosquito, sporozoites are released and they infect the salivary glands.

Mosquitoes become infectious to human during the next blood meal once the salivary gland becomes infected (Han *et al.*, 2000). About 10% of *Anopheles* mosquitos are important vector for human malaria among more than 400 species. Differences in susceptibility to *Plasmodium* infection among the putative vectors of malaria in the Amazon have never been fully and carefully considered. In Asia and Africa, mosquito vectors of malaria are present in colonies and can be maintained in the laboratory. For example, *Anopheles gambiae*, the major vector in several African countries, is the most studied mosquito, particularly with regard to its interaction with human and murine *Plasmodium* species causing malaria (González-Lázaro *et al.*, 2009).

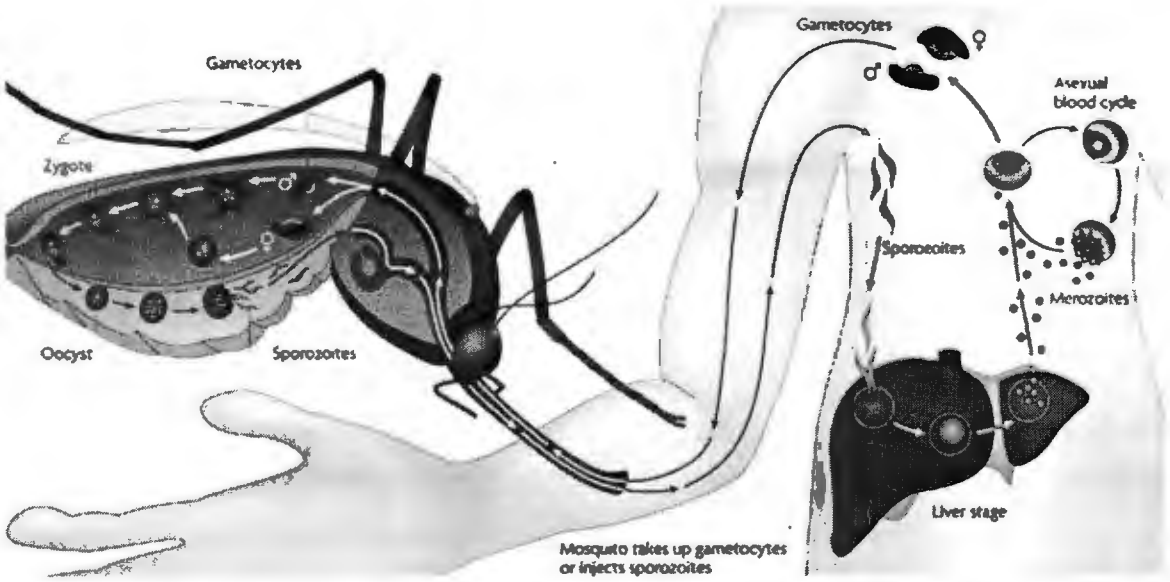


Figure 1.3 The life cycle of Plasmodium consisting of three different phases, with each phase comprising its own different stages. (http://www.roitt.com/elspdf/plasmodium.pdf).

Methods, based on the polymerase chain reaction for the dection of the DNA of parasites have been developed. However, due to the cost and complexity these methods have not been used in areas where malaria is common (Singh *et al.*, 2007). Both tropical and subtropical regions around the broad band of the equator, which include the Sub-Saharan Africa, Asia, and Latin America, are affected by malaria (Hunter *et al.*, 2007).

The World Health Organization (WHO) estimates that in 2014, an estimated 3.3 billion people are at risk of being infected with malaria and developing diseases worldwide in 2014, and approximately 1.2 billion are at high risk (>1 in 1000 chances of getting malaria in a year). In 2012, about 207 million cases of malaria were reported. It is estimated that malaria killed the people between 0.4 and 0.8 million. Many of them are children in Africa (Figure 1.4). Malaria can cause poverty and it negatively effects the economic development of the country (Worrall *et al.*, 2005). In Africa, due to the the increased healthcare cost, loss of ability to work and the negative effect on touraism, 12 billion dollor (USD) per annum are lost by the malarial infection (Greenwood *et al.*, 2005).

Non-*Falciparum* malaria is caused by *P. ovale, P. vivax*, subspecies, *P. knowlesi, or P. malariae*. Data are limited regarding the transmission and control patterns of non-*Falciparum* malarias. In the Americas and Asia, where the transmission of malaria is usually low and seasonal, *P. vivax* and *P. falciparum* malaria have nearly the same preponderance, *P. ovale* and *P. malariae* are primarily found in the sub-Saharan Africa and constitute < 10% of isolates. Currently, non-*Falciparum* malaria comprise approximately 25% of all imported 11,000 cases of malaria in Europe per year (Su *et al.*, 2007). The first-line treatment for uncomplicated *Falciparum malaria* is Artemisinin combination therapy (ACT), it is the primary recommendation. This therapy is recommended for *P. knowlesi* and traveller's malaria in endemic areas, whereas chloroquine (CQ) is still the standard drug for *P. ovale*, *P. malariae* and *P. vivax* in most countries (Obiol-Pardo *et al.*, 2011).

Different treatment recommendations may have several drawbacks in the clinical practice for the treatment of *non-falciparum* and *P. falciparum* malaria. More importantly, the misclassification of *Plasmodium* spp. is very common whenever non-*falciparum*

infections are involved, due to the variations within morphological characteristics; between and within the species and the lack in microscopy (Xue *et al.*, 2013). There may have severe or even fatal consequences due to the widespread resistance offered by *P. falciparum* to CQ during the CQ treatment for the misclassified *P.falciparum.* Even in experienced laboratories discrepant results between PCR and microscopy can still happen is spite of the internal and external quality appraisal for parasitological diagnosis (Labute *al.*, 2008). Moreover, the infections that are common are of mixed – species, but the diagnosis of this condition is specifically difficult microscopically (Croft *et al.*, 2011). Quick diagnostic tests, such as NOW® ICT MALARIA *P.f/P.*v. test based on *P. falciparum* HRP2/aldolase, cannot safely separate single-species *P. falciparum* infection from concurrent *P. ovale* or *P. malariae* (Wallace *al.*, 1995). The failure of these tests is probably due to the low quantities of antigen circulationg caused by less parasitemia in *P. ovale* and *P. malariae.* Evaluation of three rapid diagnostic tests (Paramax-3, BinaxNOW® Malaria and OptiMAL-IT) for the detection of *P. knowlesi* infection demonstrated low sensitivity (Paramax-3 RDT, 40%; BinaxNOW®, 29%; and OptiMAL-IT, 71% for fresh *P. knowlesi* samples), low specificity, and a risk of misdiagnosis of *P. vivax* or *P. falciparum.* Although PCR can univocally differentiate between subspecies, the use of this technology is still not in routine in clinical care.

P. vivax which shows resistance to CQ is coming forth in areas of South-East Asia and the sub Saharan Africa (Hsia *et al.*, 2015). Latest analysis of high order demonstrates that CQ resistance in 58 out of 113 non-exempt study sites spreads through most countries which are endemic to *P. vivax* (Gong *et al.*, 2006). The World Health Organisation also suggests the use of ACT in the treatment of *P. vivax* in areas which are affected. To date, resistance of P. malariae and P. ovale to CQ has been reported just once from Indonesia (Qian *et al.*, 2009).

Figure 1.4 The Geographical distribution of Malaria, the World Health Organization estimates 2012 (http://www.who.int/healthinfo/global_burden_disease/estimates/en/index1.html).

At last, the ingestion of CQ is very unorthodox in many African descendants because of the frequently felt side effects, such as puritus, which is caused by the affinity of CQ to melanocytes (Lipton, 2004). Artemisinin derivatives are usually well abided, and the safety profile of ACT might be largely determined by the drug associated as partner. The clinical effect caused by *non-falciparum* malaria infection happens to be more essential than that in the previous three decades. A systematic approach and high level analysis showed *P. vivax* as the major cause of severe malaria and a comparable incidence of severe malaria between *P. falciparum* and *P. vivax* was found in children, adults and infants (Sinka *et al.*, 2012).

The increasing resistance of parasites to inexpensive drugs and mosquitoes to insecticides has created an urgent need for innovative methods that block parasite transmission during their development within the insect. *Anopheles* mosquitoes not only carry the parasite from infected to uninfected people, but also play a vital role in the life cycle of the parasite. Mosquito saliva and salivary glands are central components of the interaction between the

parasite, vector, and mammalian host. As sporozoite maturation in the mosquito salivary glands before its transmission to vertebrates increases the ability of sporozoite to infect vertebrate hepatocytes, it is considered a key stage for effective transmission to humans (Eisenreich *et al.*, 2004). Furthermore, sporozoites are injected into the vertebrate skin with nanoliter volumes of saliva, a complex biologically active solution, which, in addition to other activities, serves as the "transmission fluid" for the malaria parasite. The salivary glands and their diversified protein contents are essential to overcome the challenges posed by the host, such as pain and itch responses, immune defenses, and hemostasis. Evidence has shown that the pharmacological activity of the arthropod saliva affects pathogen transmission. Salivary gland lysates from the sand fly *Lutzomia longipalpis* facilitate the infection of mice by the protozoan parasite *Leishmania major* (Xue *et al.*, 2013). However, a few studies have been conducted on the role of mosquito salivary gland proteins in promoting the infection of *Plasmodium* spp. in vertebrate hosts. During the last 5 years, several studies were performed on the sialome of *Anopheles* mosquitoes (Jakalian *et al.*, 2002). Researchers identified 67 proteins from *A. gambiae* salivary glands, an initial step toward the cataloging of the hundreds of proteins and peptides in the salivary proteome. However, no study has attempted to determine the proteome of *A. gambiae* saliva in the presence of malaria parasites. (Gong *et al.*, 2006). Importance of antimalarial drugs study was carried out with the following objectives.

1. The present thesis deals with the discovery of lead compounds against this enzyme by performing in-silico screening of novel antimalarial compounds with it structure.
2. Beta-hydroxyacyl-acyl carrier protein dehydratase (FabZ) from Plasmodium falciparum has been reported as the drug target for antimalarial drug fosmidomycin. FabZ is an enzyme in the fatty acids biosynthesis pathway. It is essential for the pathogens and is absent in human, which makes it a good target for drug discovery.
3. This study demonstrates the identification of the 35 molecules as potential candidates for the drugs against b-hydroxyacyl-acyl carrier protein dehydratase (FabZ), using structure aided virtual screening tools and databases. Molecules have been characterized with the binding energy. Given the good affinities of these compounds to the target proteins, they have good potential to be developed into to good drugs against malaria in the future.

CHAPTER NO: 2

LITERATURE REVIEW

Review of Literature

Malaria is one of the world's most common and serious diseases causing death of about 3 million people each year. Its most severe occurrence is caused by the protozoan *Plasmodium falciparum*. There are numerous factors that contribute to the persistence of malaria. Vector control is hampered by financial constraints and insecticide resistance. Treatment programs are limited by the poverty of most endemic areas, and despite enormous efforts an effective malaria vaccine is still not available. The anti-malarial treatment has depended on drugs developed decades ago, and the occurrence of resistance against almost all available drugs has largely contributed to the recent resurgence of malaria. Biomedical research could enable treating the disease by effectively and specifically targeting essential enzymes of this parasite. However, the parasite has developed resistance to existing drugs making it indispensable to discover new drugs. The recent successful completion of the genome sequencing of *Plasmodium falciparum*, the causative agent for the most severe form of malaria, has been a milestone that provides a tremendous amount of information on a genetic level (Gardner *et al.*, 2002).

A series of highly promising and so far unknown or only scarcely described metabolic pathways in *P. falciparum* were identified through the genome project, among which a complete type-II fatty acid biosynthesis system (FAS-II) could be detected (Gardner *et al.*, 2002). The FAS-II pathway represents a particularly interesting drug target, as there are major differences between the structural organization of the plastid-associated enzymes found in plants and most microorganisms including *Plasmodia*, and the cytosolic enzymes of mammals and yeast (Rock and Cronan 1996). The discovery of a series of compounds (e.g., thiolactomycin, diazaborines, isoniazid, triclosan) that can selectively inhibit FAS-II pathways served to validate FAS-II enzymes as drug targets (Waller *et al.*, 2003). The third step in chain elongation during fatty acid biosynthesis in *P. falciparum* is carried out by the β-hydroxyacyl-ACP dehydratase (PfFabZ) and corresponds to the primary dehydratase (EcFabZ) participating in fatty acid biosynthesis of *E. coli*. These findings clearly demonstrated the essential role of fatty acid biosynthesis, and it could be shown by us and others that *P. falciparum* FAS-II may comprise very attractive novel targets for the development of new and selective anti-malarial drugs. (McLeod *et al.* 2001). In the recent studies we have established a simple computational tools, which analyses the topology of the

metabolic network of *P. falciparum* to identify essential enzymes as possible drug targets. We investigated the essentiality of a reaction in the metabolic network by inhibition such a reaction *in silico.*

2.1 Computer Aided Drug Development

The searches for new effective and safe drugs are increasing day by day. The discovery of new drugs takes a time of several years and a billion of dollars are required plus human resources are also needed. Recent research shows that the discovery of new drugs take approximately a time of several years and a millions of dollars are spent (Kapetanovic *et al.*, 2008) and about many percent this rate has been increased every year. A big challenge to produce efficient drug is similar as that was earlier i.e. to minimize the charges, to reduce the time required and make lessen the risk. Beside this a thousands of chemical compounds were tested, among which few will come under medicinal study (Ekins and Wang 2006). So it means that a large number of chemical compounds were tested biologically to obtain hit compound which were further proceed to get ideal compound which is further tested. To overcome this problem, many computer technologies and approaches have been introduced which help in reducing the time period as well as high cost during drug discovery (Hann and Oprea 2004).

The rapid advances in bioinformatics help us in better understanding of drug and target interactions and thus this interaction plays a main role in introducing a computer technology, CADD (Computer Aided Drug Design), the other names used in this perspective is rational drug design, *in-silico* drug design etc. (Jain, 2003; Jain, 2004; Kumar *et al.,* 2006; Oprea and Matter, 2004; Roche and Guba, 2005; Stahl *et al.,* 2006; Stoermer, 2006). The idea of cost reduction and to lesser the time period to produce drugs is fulfilled by combinatorial chemistry (Myers, 1997). A big numbers of molecules are screened in a systematic manner by using the tools provided by combinatorial chemistry. Tools use plays an important and significant role in the designing and the invention of clinically important chemical entities. Computer software used molecular techniques to design the initiative steps to increase the molecular diversity to virtually synthesize the chemical libraries (Gallop *et al.,* 1994; Gordon *et al.,* 1994). In short the CADD overcomes

the problem come in the way of drug discovery like, large amount of money to invest, human resources plus a big time period.

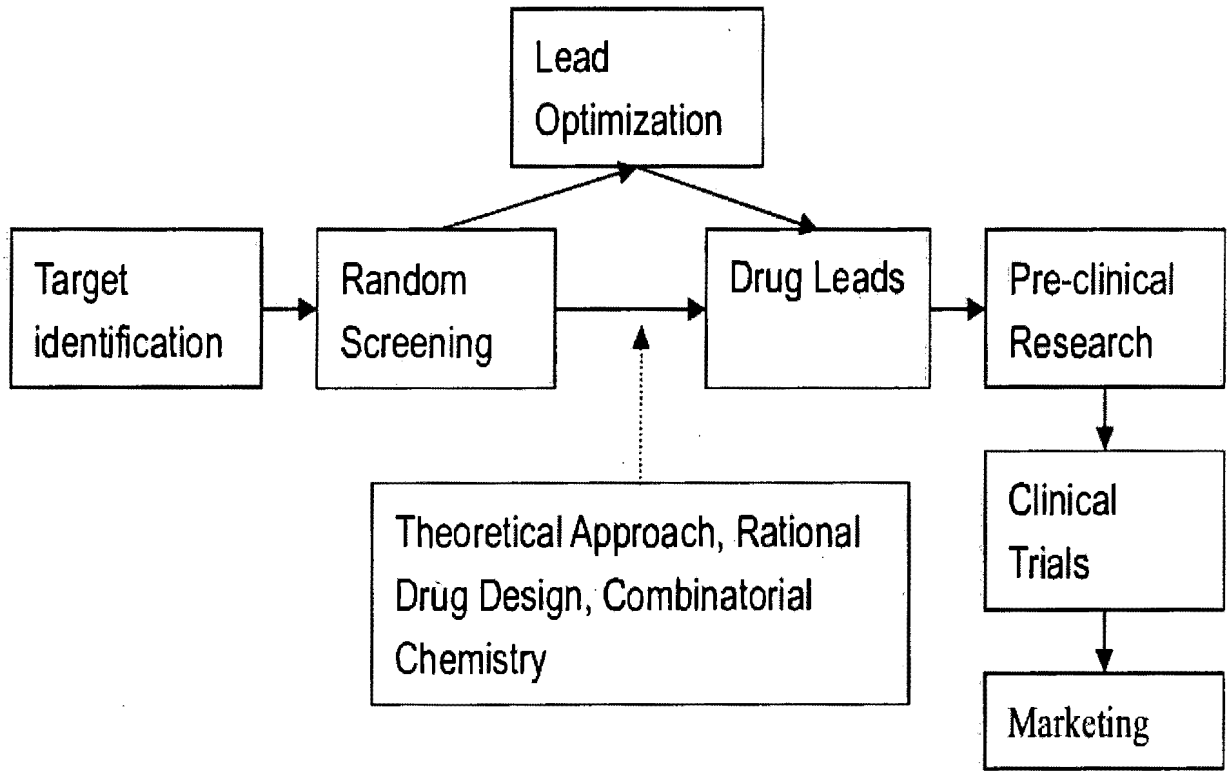


Figure: 2.1 Stages of the new drug discovery process.

2.1.1 Molecular Docking

For the accuracy of lead compounds discovery it is essential to give importance to those chemicals that are known to be an ideal drug leads. If the appropriate drug target and its 3D structures are known, SBDD (structure based drug design) can be performed. By using the NMR techniques and x-ray crystallography structures of many drug targets have been developed (Vesolovoski and Ivinov, 2003) based on geometric and chemical complementarities to ligand-target complex to match the expected binding affinity. Docking and Denovo design are the two distinct techniques used in SBDD. Systematic and stochastic approaches are the two algorithms used to dock ligands in SBDD. The basic difference between them is that systematic approach involves the rebuilding the structure of ligand within the active site of receptor. While that of the stochastic approach the ligand structure is dealt from the initial step. Docking is generally used for the screening of potent compounds or that of a compound which is medicinally important and is an ideal compound from huge available database. Denovo design evolves the construction of ligands utilizing the target structural information (Colman, 1994).

The purpose of docking methodologies is to forecast the ligand and target complex and to align the molecular databases on the basis of binding affinity to that of target (Jones, 1995; Kuntz *et al.,* 1994; Lenguer and Rarey, 1996; Lybrand, 1995; Rosenfeld *et al.,* 1995). Various methods are used in docking a ligand to the target. These are MOE-Dock (Chemical computing group, 2010), Gold (Halgren *et al.,* 2004), DOCK (Kuntz, 1992), Auto Dock (Moris, *et al.,* 1998), Surflex (Jain, 2003) and Tripos FlexX (Rarey *et al.,* 1998).

Beside ligand-protien docking another type of docking is also carried out i.e. protein-protein docking, for this purpose the method of 3D dock (Katchalski-Katzir *et al.,* 1992) (FTDOCK) is used. These all methods are different from each other because of having difference in atomic partial charges parameters as well as the force field.

2.1.2 Molecular Dynamics Simulations:

Molecular dynamics simulations are important tools for understanding the physical basis of the structure and function of biological macromolecules. The early view of proteins as relatively rigid structures has been replaced by a dynamic model in which the internal motions and resulting conformational changes play an essential role in their function. Current computer simulations of biomolecules typically make use of classical molecular dynamics methods, as a very large number (tens to hundreds of thousands) of atoms are involved over timescales of many nanoseconds

2.2 Antimalarial medication

Malaria can be prevented or cured by the antimalarial medications (also called antimalarial agents). Such antimalarial agents are used for the following cases:

I. Treatment of patients having confirmed or suspected infection.
II. Prevention of infection in that individuals who is visiting the malaria-endemic region with no immunity
III. Routine treatment on an intermittent basis to certain groups in endemic regions
IV. Rheumatoid arthritis and lupus-associated arthritis can also be treated by CQ and hydroxychloroquine.
V. Treatment solely based on clinical suspicion should only be considered when a parasitological diagnosis is not accessible (Reyburn *et al.*, 2010).

2.2.1 Quinine and related agents

Quinine has a long history stretching from Peru, ranging from the discovery of the cinchona tree and the potential uses of its bark to the current day and collection of derivatives that are still frequently used in the prevention and treatment of malaria. Quinine is an alkaloid that acts as blood schizonticidal and weak gametocide against *P. vivax* and *P. malariae*. As an alkaloid, quinine accumulates in the food vacuoles of *Plasmodium* species, particularly *P. falciparum*.

Quinine facilitates the aggregation of cytotoxic heme and thus inhibits hemozoin biocrystallization. Although quinine is less effective and more toxic as a blood schizonticidal agent than CQ, it is still very effective and widely used in the treatment of acute cases of severe *P. falciparum*. Quinine is specifically useful in areas with a high level of resistance to CQ, mefloquine, and sulfa drug combinations with pyrimethamine; quinine is also used in post-exposure treatment of individuals returning from an area where malaria is endemic (Jones *et al.*, 2015).

The use of quinine is characterized by frequently experienced syndrome called cinchonism. The most common symptoms include tinnitus (hearing impairment), nausea, rashes, vomiting, abdominal pain and vertigo.

Neurologic effects are experienced by the malarial patients in some cases due to the neurotoxic effect of the drug. Due to the interaction of the quinine, the neurologic effects are mediated and thus cause a decrease in the excitability of the motor neuron. This phenomenon is often associated with the loss of function of the eighth cranial nerve and thus results in the coma, confusion and delirium. Therapeutic doses of quinine can cause hypoglycemia by stimulating insulin secretion; therefore, observation of the glucose level in patient should be done every four to six hours. In pregnancy, the dosage of drug should be monitored because the hypoglycemic affects is hypoglycemia women. Death and renal failure can occur through depression of the respiratory system due to the repeated or over-dosage.

In the treatment and prevention of malaria, the Quinimax and quinidine are mostly used alkaloids. Quinimax contains four alkaloids that are cinchonidine, quinine, cinchoine and quinidine. Because of synergistic action of four alkaloids, this combination is considered to be most effective than quinine. The other derivative such as Quinidine (the direct derivative of quinine) is the distereoisomer of quinine and has been proofed to have the same antimalarial activity (Bezati *et al.*, 2015).

Quinidine is the only drug recommended for the severe case of malaria. In 1834, Dr. Carl Warburg developed Warburg's tincture, which included quinine as the key ingredient. In the 19th century the well-known antimalarial drug was quinidine. Although originally sold as a secret

medicine, Warburg's tincture was highly regarded by many eminent medical professionals who considered it as being superior to quinine (such as Surgeon-General W. C. Maclean, Professor of Military Medicine at British Army Medical School, Netley). Warburg's tincture appeared in Martindale, with the complete drug reference from 1883 until about 1920, and was published in the Lancet 1875 (Henriques *et al.*, 2015).

2.2.3 CQ (Chloroquine)

CQ was, until recently, the most widely used antimalarial drug and the original prototype from which most methods of treatment are derived. This drug is also the least expensive, best tested, and safest of all available drugs. The emergence of drug-resistant parasitic strains rapidly reduces its effectiveness; however, CQ remains the first-line drug of choice in most sub-Saharan African countries. To extend the effectiveness of CQ, its usage with other antimalarial drugs has been suggested. The use of CQ in combination with other antimalarial drugs to extend its effective usage has been suggested. Popular drugs based on CQ phosphate (also called nivaquine) are CQ FNA, Resochin, and Dawaquin (Petersen *et al.*, 2015).

CQ is a 4-aminoquinolone compound. It mechanism of action is very complicated and is unclear. This compound is believed to reach high concentrations in the vacuoles of the parasite, resulting in increased internal pH caused by its alkaline nature. By inhibiting the bio crystallization of hemozoin, the conversion of toxic heme to hemozoin is controlled by the CQ. By this action, the parasite will poisoned by the excess levels of toxicity. CQ may act with other potential mechanism such as the formation of a CQ-heme complex, interfering with the biosynthesis of parasitic nucleic acids and CQ-DNA complex formation.

The most significant level of activity is against all forms of the schizonts (with the obvious exception of CQ-resistant *P. falciparum* and *P. vivax* strains) and gametocytes of *P. vivax*, *P. malariae*, and *P. ovale*, as well as the immature gametocytes of *P. falciparum*. CQ also exhibits significant antipyretic and anti-inflammatory effects when used to treat *P. vivax* infections, and thus remains useful even when resistance is widespread. According to a report on the Science and Development Network website's sub-Saharan Africa section, minimal drug resistance is present among children infected with malaria on the island of Madagascar, although they exhibit drug resistance against CQ (Farias *et al.*, 2015).

For many years, CQ has been used for the treatment of malaria. During this time, no teratogenic and abortifacient effects have been reported. Therefore, during pregnancy this drug is very safe to use for the treatment. However, itching can occur at intolerable levels and CQ can provoke psoriasis

2.2.4 Amodiaquine

Amodiaquine, a 4-aminoquinolone antimalarial drug, has a similar structure and mechanism of action to CQ.

. In case of CQ resistance, Amodiaquine has tended to be administered. In some patients Amodiaquine has administered because it has lower tendency to cause itching than CQ. This drug is now available in a combination with artesunate and recommended by the WHO. The combination with sulfadoxine and pyrimethamine is no longer recommended (Falajiki *et al.*, 2015).

2.2.5 Pyrimethamine

Pyrimethamine is used for the treatment of uncomplicated malaria. It has been considered to be useful in combination with sulfadoxine in case *P. falciparum* resistance to CQ. Pyrimethamine halts the cell division, reproduction and the processes of DNA replication by the blocking the pyrimidine and purine biosynthesis through the inhibition of dihydrofolate reductase in the parasite. Pyrimethamine primarily functions on the schizonts during the erythrocytic phase, and this drug is currently used in combination with sulfonamide (Artimovich *et al.*, 2015).

2.2.6 Proguanil

In 1945 Proguanil (biguanide), a synthetic derivative of pyrimidine was developed by a British antimalarial research group. It is mediated by the conversion to the cycloguanil (an active metabolite of Proguanil). Proguanil halts the cell division, reproduction and the processes of DNA replication by the blocking the pyrimidines and purines biosynthesis through the inhibition of dihydrofolate reductase in the parasite. This effect is prominent in primary tissue stages of *P. ovale, P. falciparum* and *P. vivax.*

This drug cannot be used to prevent relapse because it has no know effect against hypnozoites. It cannot be recommended for acute infection therapy due to its low blood schizonticidal activity. For prophylaxis, Proguanil is used in combination with atovaquone or CQ. A considerable level of protection is provided by this drug due to its pharmacokinetic profile. It indicates that significant level of consistency in plasma can be maintained by the half dose used two times in a day. The combination of proguanil with CQ has no effect on resistant strain of *P. falciparum.* Mouth ulcers and slight hair loss are few side effect reported. Proguanil hydrochloride is marketed as Paludrine by AstraZeneca (Grynberg *et al.*, 2015).

2.2.7 Sulfonamides

Sulfadoxine and sulfamethoxypyridazine block the tetrahydrofolate synthesis pathway through the inhibition of dihydropteroate synthetase in malarial parasites. The structures of these compounds are analogs of p-aminobenzoic acid. It blocks the conversion of p-aminobenzoic acid to dihydrofolic acid by competing wih p-aminobenzoic acid. Sulfonamide is active against the schizont stages the erythrocytic cycle (asexual). Sulfonamide is not effective against malaria when used alone. It combination with antifolate pyrimethamine have a synergistic effects against malarial parasites. It common fixed dose is sulfadoxine-pyrimethamine (Fansidar). Due to skin reaction Sulfonamide is not recommended for chemoprophylaxis. Nevertheless, sulfonamides are frequently used for clinical episodes of the disease (Zali-Boeini *et al.*, 2015).

2.2.8 Mefloquine

Mefloquine, which is chemically related to quinine, was developed during the Vietnam War to protect American troops against the multi-drug resistant *P. falciparum.* Mefloquine is a very potent blood schizonticide with a long half-life. It forms a toxic heme complex that damages the food vacuole of parasite. Despite being active against *P. malariae, P. vivax* and *P. vivax,* it can also be recommended against the malarial strain that is resistant such as *P. falciparum.* In case of prophylaxis and acute therapy, Mefloquine is very effective. It is strictly used for the resistant strain combined with artesunate. For all other *Plasmodia* infections, the combination of CQ/proguanil or sulfa drug–pyrimethamine is used. Esophagitis and vomiting are most reported in children with increased dosage of this drug. During the first trimester Mefloquine should not be administered while the second and third trimesters, it is considered to be safe. In October

2011, the Centers for Disease Control and Prevention changed its recommendation. They approved that during trimester mefloquine can be used for both prophylaxis and treatment of malaria. Dizziness, vomiting, diarrhea, abdominal pain and nausea are reported as side effects of Mefloquine. This drug is also associated with neurological events, such as affective and anxiety disorders, hallucinations, sleep disturbances, psychosis, toxic encephalopathy, convulsions, and delirium. Among the patients that are treated with mefloquine 68% of them experience cardiovascular effects with bradycardia and sinus arrhythmia.

Because of its side effects Mefloquine can be used for a maximum period of 6 months. Other drugs (such as those based on paludrine/nivaquine) should be subsequently administered (Hopperus Buma *et al.*, 1996).

2.2.9 Atovaquone

Atovaquone is available in market in combination with proguanil. It is sold under the name Malarone with high price than Lariam. For travelers, this drug can be used for prophylaxis. This drug is also used to treat malaria caused by *P.falciparum* in developed coutries. Mepron is the liquid oral suspension of atovaquone available in market (Guler *et al.*, 2015).

2.2.10 Primaquine

Primaquine is used for the treratment of all type of malarial. Primaquine is a highly active 8-aminoquinolone. Primaquine is most active against gametocytes. This drug can also act as a blood schizonticytes, hypnozoites and the dormant plasmodia in *P. ovale* and *P. vivax.* For the treatment of acute cases and relapsing malaria infections, Primaquine is only known drug. The mechanism of action is not fully understood, but it is assumed to block oxidative metabolism in *Plasmodia* (Weaver & Lieberman, 2015).

2.2.11 Artemisinin and derivatives

Artemisinin, a Chinese herb, over 1000 year it has been used for the treatment of fevers. Artemisinin is extracted from the plant *Artemisia annua.* In 340 AD, Ge Hong discovered for the first that this compound is the successful therapeutic agent for the treatment of malaria. He documented this in his Zhou Hou Bei Ji Fang (A Handbook of Prescriptions for Emergencies). Ge Hong used a simple macerate to extract artemesinin and this method is still used nowadays.

In 1971, the active compound artemsinin was isolated. It is a sesquiterpene lactone with a chemically rare peroxide bridge linkage. Thus artemisinin is considered to have antimalarial activity. The target of this compound within parasite is remains controversial. At present, this drug has been approved to have antimalarial activity against all type of multidrug resistant *P. falciparum.* This drug is now controlled by WHO. Artemisinin can also be administered in combination with other drugs (Zang *et al.*, 2015)

Majority of the acute patients show significant improvement when treated with Artemisinin for one to two days because Artemisinin has a very fast action. The drug has the fastest clearance of all antimalarial agents currently used and acts primarily on the trophozite phase, thus preventing the progression of the disease. The semi-synthetic derivatives of artemisinin artemether and artesunate are converted into its active form dihydroartemesinin rapidly when administered into the body. These derivatives are easy to use than parent compound.

Artesunate is a hemi succinate derivative of the active metabolite dihydroartemisin. Currently, this drug is the most frequently used of all the artemesinin-type drugs and its effect is mediated through reduced gametocyte transmission. Artesunate is used in combination therapy. It has been proved that this drug is active in case of uncomplicated *P. falciparum.*

Arteether is an ethyl ether derivative of dihydroartemisinin used in combination therapy in case of uncomplicated resistant *P. falciparum* (Ho *et al.*, 2015).

2.2.12 Halofantrine

In 1960, Walter Reed Army Institute of Research developed a new drug Halofantrine. Chemically this drug is phenanthrene methanol and related to quinine. It is active against all plasmodium parasites as a blood schizonticide. Its mechanism of action is same as other antimalarial drugs. It damages the plasmodial membrane due to the formation of cytotoxic complexes with ferritoporphyrin XI. Due to its high cost, it is not recommended for the treatment of therapeutic and prophylactic malaria despite being active against drug-resistant parasites. Moreover, halofantrine has very variable bioavailability and potentially high levels of cardio toxicity. However the patient that has no heart disease and is suffering from resistant and acute malaria uses this drug. Halfan is a popular drug based on halofantrine. The level of governmental

control and the prescription-only basis on which it can be used contributes to the cost; thus, halofantrine is not frequently used.

For children, who have weight lower than 10 kilogram, Halofantrine is not recommended. Itching, nausea, abdominal pain and diarrhea are the side effect most frequently experienced by the patients treated with this drug. Due to high dose, ventricular dysrhythmias can be experienced that cause death. During pregnancy and lactation, Halofantrine should not be recommended. It should not be used by the patients who already took mefloquine. Lumefantrine is a relative of halofantrine and is used in some combination antimalarial regimens (van Vugt *et al.*, 1998)

2.2.13 Doxycycline

Doxycycline is an antimalarial drugs derived from oxytetracycline. Chemically this is tetracycline compound. This is the most prevalent drugs due its effectiveness and cheapness. Tetracycline is included in of the earliest developed groups of antibiotics. Tetracyclines are still used for many type of infection. Being a bacteriostatic agent, this drug prevent the bonding of of 50s and 30s ribosomes by the binding to the 30S ribosomal subunit thus inhibit the protein synthesis. In Areas where with CQ resistance, Doxycycline is used for chemoprophylaxis. In combination with quinine, it can be used to treat resistant *P. falciparum.* In acute malaria this drug has very slow action so, it cannot be used as monotherapy.

For acute malaria, due to its slow onset Tetracycline is only used in combination. Unlike doxycycline, this drug is not used in chemoprophylaxis. Esophageal ulceration, gastrointestinal upset, interferences with the process of ossification, and depression of bone growth are known to occur. The majority of side effects associated with doxycycline are also experienced (Panic *et al.*, 2014).

2.2.14 Clindamycin

Clindamycin is derived from lincomycin. It has a slow against blood schizonticides. For the treatment of malaria caused by resistant *P. falciparum* this antibiotic can be used in combination with quinine. It is very expensive and toxic than other antibiotics so it should be administered only when tetracycline are contraindicated. Nausea, vomiting, and abdominal pains and cramps

are the only side effects recorded in patients taking clindamycin. However, by drinking too much water these side effects can be alleviated while using this drug. Pseudomembranous colitis (caused by *Clostridium difficile*) has also developed in some patients, and this condition may be fatal in a small number of cases (Na-Bangchang *et al.*, 2006).

CHAPTER NO: 3

MATERIALS AND METHODS

3. Materials and Methods

3.1 Receptor Protein preparation

The initial coordinates of β-hydroxyacyl-ACP dehydratase (PfFabZ) complex with *Pyridine-containing Inhibitors* of *Plasmodium falciparum* with PDB ID 4GAE, was retrieved from PDB databank. All the crystal water molecules were deleted in the starting model. The ff14SB and gaff force field were used to produce the parameter for the protein and inhibitor. The partial atomic charges was assigned to inhibitor by using the am1-bcc method implemented in the amber14 (Jakalian *et al.*, 2002).

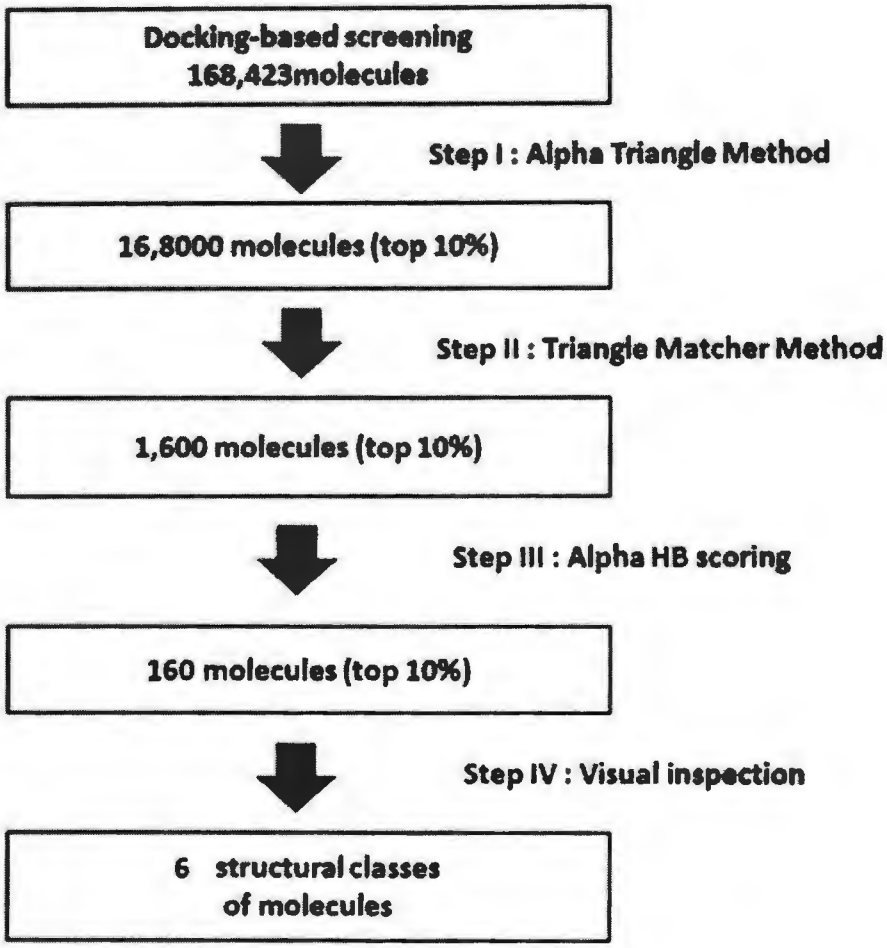
The system was solvated in the octahedral box of TIP3P water molecules with 8.0 Å buffer along each side. Subsequently the system was made neutral by adding the sodium counter ions. In order to refine the initial coordinates of complex, a simulation of 50 nanoseconds was performed using the amber14 software. The final coordinate after MD simulation reflects the native structure of the complex. Further docking study was done with the final coordinate after MD simulation.

3.2 Ligand database at http://www.chembridge.com/index.php

The ChemBridge database was used as the ligand library that includes 168423 compounds (http://www.chembridge.com/screening_libraries/). The energy of chemicals in the database was minimized by using the Energy Minimized program implemented in MOE.

3.3 Molecular Docking

The Moe-Dock program implemented in MOE2014 was used for the docking purpose. The binding site, defined by the *Pyridine-containing* Inhibitor, was used for the docking of ligands. We developed a protocol for the docking-based screening and searched Chembridge commercial database using three successive hierarchical docking filters (Figure 3.1).



v

Figure 3.1: Moe-Dock program implemented in MOE2013

3.3.1 The Alpha Triangular Method

The alpha triangular method was used as a docking protocol to retrieve the molecule from the ChemBridge database with good affinity towards the target protein. The alpha triangle method generates the poses by the superposition of ligand triplets and triplets of receptor site protein. The receptor site points were alpha sphere centers which represent locations of tight packing. For each ligand five poses were generated. At the end of this step, the docked molecules were sorted on the basis of docking score (S-score). The more negative value of the S-score shows the most favorable binding pose of molecule in the binding pocket. The docked molecules having the lowermost S-score than the experimental verified inhibitors were chosen for the

further study. Total of 16, 8000 molecules having low S-score were selected from the ChemBrigde database.

3.3.2 Triangle Matcher Method

The triangle matcher method generates poses by aligning ligand triplets of atoms on triplets of alpha spheres in a more systematic way than in the alpha triangle method. The docked molecules having the lower S-score than the experimental verified inhibitors were chosen for the further study.

3.3.3 Alpha HB Scoring.

This score is a linear combination of two terms. The first term measures the geometric fit of the ligand to the binding site. The second term measures hydrogen bonding effects. The poses generated by Alpha HB scoring were refined on the basis of force field refinement and rescored using Generalized-Born Volume Integral/ Weighted Surface Area GBVI/WSA dG scoring function. The GBVI/WSA is a scoring function which estimates binding free energy from a given pose of the ligand. The resulted binding interactions of the given pose of the ligand (having lowermost S-score) and protein were analyzed using LigPlot implemented in MOE.

3.4 Binding Energy and Binding Affinity Calculations

All the hit compounds were further subjected for the Binding Energy and Binding Affinity Calculations for the identification of potential ligands. The binding affinities of all the hits compound were calculated with generalized Born / volume integral (GB / VI) implicit solvent method implemented in MOE (Labute *et al.*, 2008). Generalized Born interaction energy, such as Vander Waals, implicit solvent interaction energies and Coulomb electrostatic interaction, is non-bonded interaction energy between the receptor and the ligand molecule. The atoms of the receptors that are away from the active pocket were kept fixed, and the active pocket along with the ligand were kept flexible but were subjected to tether restraints that discourage gross movement. The ligand atoms were set free to move at the binding pocket. The energy minimization of the PfFabZ-ligand complex was carried out in each case before calculating binding affinity. The binding affinity was calculated for each hit after energy minimization and

reported in unit of (KJ/Mol). The complexes having good affinity toward the PfFabZ were further subjected to MD simulation to check its stability.

3.5 Ligplot analysis

Ligplot is a computer program that generates schematic 2-D representations of protein-ligand complexes from standard Protein Data Bank file input (Wallace *et al.*, 1995). The Ligplot is used to generate images for the PDBsum resource that summarises molecular structure. Weak intermolecular interactions such as hydrogen bonding and hydrophobic interactions are key players in stabilizing energetically-favored ligands, in an open conformational environment of protein structures. In-silico docking studies were performed MOE and visualize using Ligplot v.4.5.3. Ligplot is used to investigate the role of top scorer ligands in the binding pocket of pfFabZ. Hydrophobic and hydrogen bonding interactions of each docked molecule were compared using LigPlot program.

3.6 Protein Data Bank

The Protein Data Bank (PDB) is a repository for the three-dimensional structural data of large biological molecules, such as proteins and nucleic acids. The data, typically obtained by X-ray crystallography or NMR spectroscopy and submitted by biologists and biochemists from around the world, are freely accessible on the Internet. The PDB is overseen by an organization called the Worldwide Protein Data Bank, wwPDB. The PDB is a key resource in areas of structural biology, such as structural genomics.

Most major scientific journals, and some funding agencies, now require scientists to submit their structure data to the PDB. (http://www.rcsb.org/pdb/home/home.do)

3.7 Molecular Operating Environment (MOE)

Molecular Operating Environment (MOE), is a comprehensive software for life and material science. It developed by chemical computing group (CCG). MOE is a package of software that implements visualization, simulation and application development in one package. MOE highly supports drug design through molecular simulation, protein structure analysis, data

processing of small molecules, docking study of proteins and small molecules, and so on under the unified operations.

(https://www.chemcomp.com/MOE-Molecular_Modeling_and_Simulations.htm).

3.8 Molecular Dynamics Simulations

The complexes having the good binding affinities were subjected to the molecular dynamics (MD) simulations using the Amber14 software. The MD simulations were carried out in order to check the stability of each derivative in the active site of each enzyme. The *ff14SB* force field was used to define the protein using the *tleap* module of AmberTools15 (Guttery *et al.*, 2012). *Tleap* was also used to add hydrogen atoms to each complex and neutralize each system with Na+ counter ions. Each system was then immersed into the rectangular box of TIP3P water molecule with a buffer distance of 8 Å (Abeles *et al.*, 2013). The accelerated GPU *pmemd* code was used to perform all steps of MD for each system. The minimization of each system was done in six steps including 1000 steps of steepest descent minimization followed by 1000 steps of conjugate gradient minimization at each step. Initially, the positional restraints on water molecules were kept 500kcal/mol/Å^2 and systematically lowered down to zero in six steps. After minimization, each system was heated up to 300K in 1 nanosecond (ns) through five steps. Initially the positional restraints on water molecules during heating were kept 500kcal/mol/Å^2 and systematically lowered down to 100kcal/mol/Å^2 in five steps. The heating was followed by density equilibration step. The density of the each system (protein and ligand) was equilibrated with weak restraints for the 5ns followed by the equilibration of the whole system at constant pressure for the 5ns. Finally the whole system was subjected to unrestrained MD simulation for the 25 ns, saving the trajectory after each 20 ps. During simulation the pressure was kept constant using Berendsen thermostat and temperature was control with Langevin thermostat (1 atm, 300K). Long-range electrostatic interactions were computed by employing Particle Mesh Ewald (PME) with the default setting in AMBER14 (PIMENTA 1994). The cutoff distances for the long range electrostatic and van der Waals interactions were set to 10.0 Å. The SHAKE algorithm was used for the covalent bonds involving hydrogen (González-Lázaro *et al.*, 2009). The trajectory of MD simulation was analyzed for the structure stability.

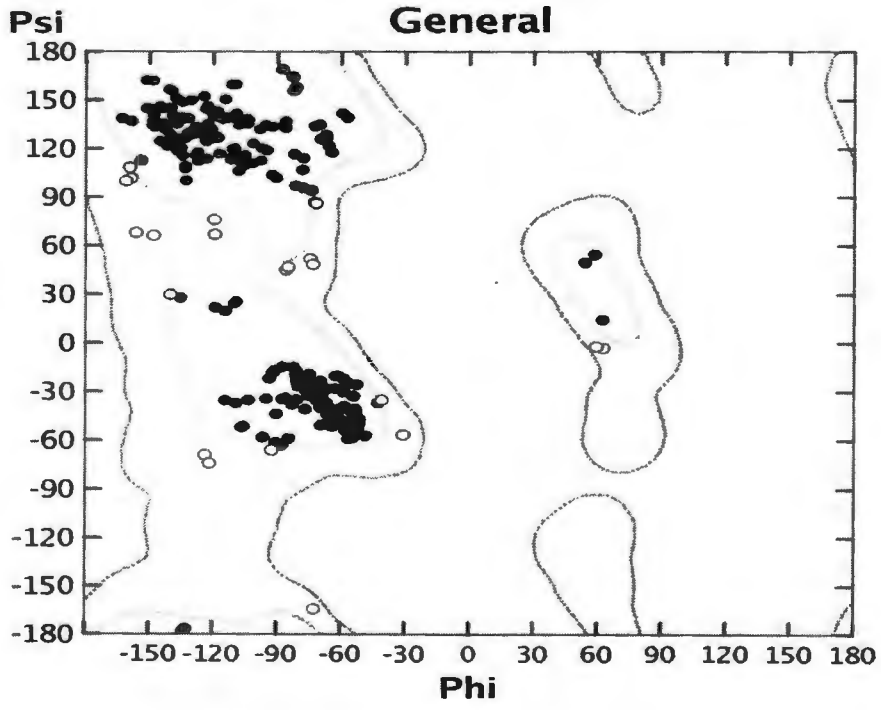
CHAPTER NO: 4

RESULTS

4. Results

4.1 Receptor Protein preparation

The X-ray crystal structure of b-hydroxyacyl-acyl carrier protein dehydratase (FabZ) of Plasmodium falciparum with PDB ID 3AZ9 was retrieve from protein databank and was used as receptor for the docking purpose. All the crystal water molecules were removed from the structure and were subjected to the MD simulation for the refinement. The b-hydroxyacyl-acyl carrier protein dehydratase (FabZ) was simulated for 30 nanosecond (ns) using amber14 software. After the MD simulation the quality of FabZ was measured in term of Ramachandran Plot and Root Mean Square Deviation (RMSD). Ramachandran Plot is the best-known, and certainly most powerful, check for the stereochemical quality of a protein is the φ-ψ Plot. Figure 4.1 shows the Ramachandran Plot of Fabz. After MD simulation no residue was found in theoutlier region suggesting the good conformation of the FabZ



4.1 Ramachandran Plot of Fabz.

Figure 4. 2 shows the RMSD of the FabZ. The RMSD were plotted as a function of time. The figure 2 shows that the RMSD raised up to 1.5 Angstrom during the first 10 ns. This shows that the structure undergoes some structural changes to remove any bad clashes between atoms. The RMSD remain unchanged all over the simulation. This shows that the structure is now stable and reasonable for the further docking purpose.

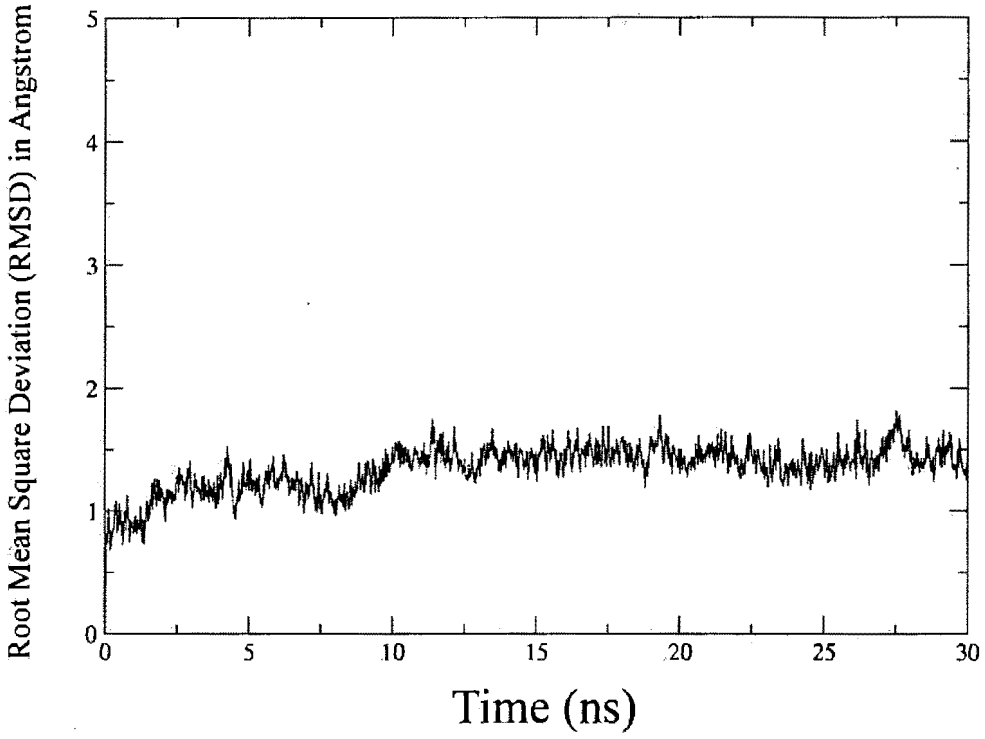
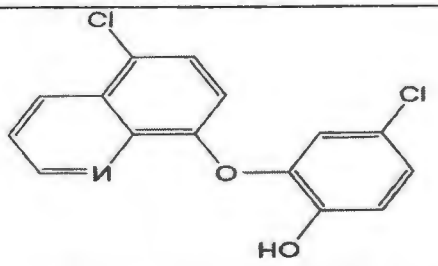
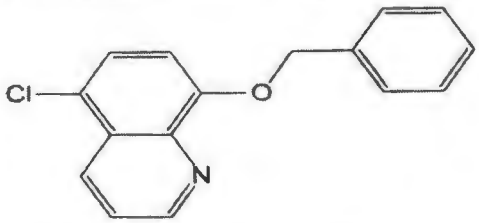
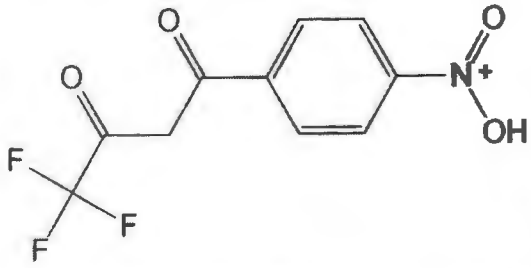
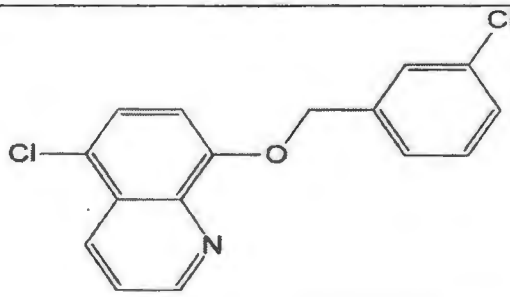


Figure 4. 2 Root Mean Square Deviation Of FabZ.

4.2Molecular Docking and Binding affinity calculation

The Resultant refined structure from The MD simulation was used as receptor for the docking purpose. The Molecular Operating Environment (MOE) software was used as docking software. The already reported inhibitors were drawn using the Builder module of MOE and docked in the active site of FabZ.

4.1 Shows the 2D structure of the reported inhibitors along with IC50 values and docking score.

Compound name	Structure	IC50 µM	Docking Score
NAS91		4.5	-13.958
NAS91-10		7.4	-13.252
NAS21		10.2	-13.037
NAS91-11		12	-12.988

The best inhibitor NAS91 has IC50 value 7.4 µM. from the docking result it has been shown that NAS51 has the highest negative docking score. This shows the reliability of the docking score. The docking score of the NAS51 was used as cutoff value to screen the chembridge database. As a results of the mentioned docking protocol, 5 compounds were found to have good docking score than NAS51.

4.2 The drug like properties and docking results of the final hit compounds are summarized in table

No	Chembridge ID	Molecular weight	Drug like Properties				MMGB/VI Kcal/mol	Affinity pKi
			logP	TPSA	don	acc		
1	10441542	350.44	1.788	62.58	3	3	-15.851	10.82
2	11235803	395.52	2.632	55.00	2	3	-13.497	10.634
3	12083893	413.85	3.795	18.18	1	5	-14.401	11.008
4	12407020	403.52	4.488	46.790	2	3	-13.128	10.723
5	12889810	395.568	1.606	66.490	3	3	-11.576	10.950
6	NAS91	612.296	10.079	84.568	2	4	-9.260	6.764

4.3Binding mode of final hits compound

The docking studies shows that final hits compounds bind into the active site with good binding energy and binding affinity. The most active compound NAS91 with IC50 value 4.5 μ bind tightly in the active site of FabZ (Figure 3). The binding score of NAS91 was found to be -13.958. Its binding affinity and binding energy were found to 6.764 pKi and -9.260Kcal/mol respectively. The NAS91 make one hydrogen bond with the active site residue His133. The other residues of the active site such as His98, Phe134, Pro141, Gly142, Val143, Glu147, Ala150, Gln151, Phe169, Leu170, Phe171 and Phe226 were found to have hydrophobic interactions (Figure 4)

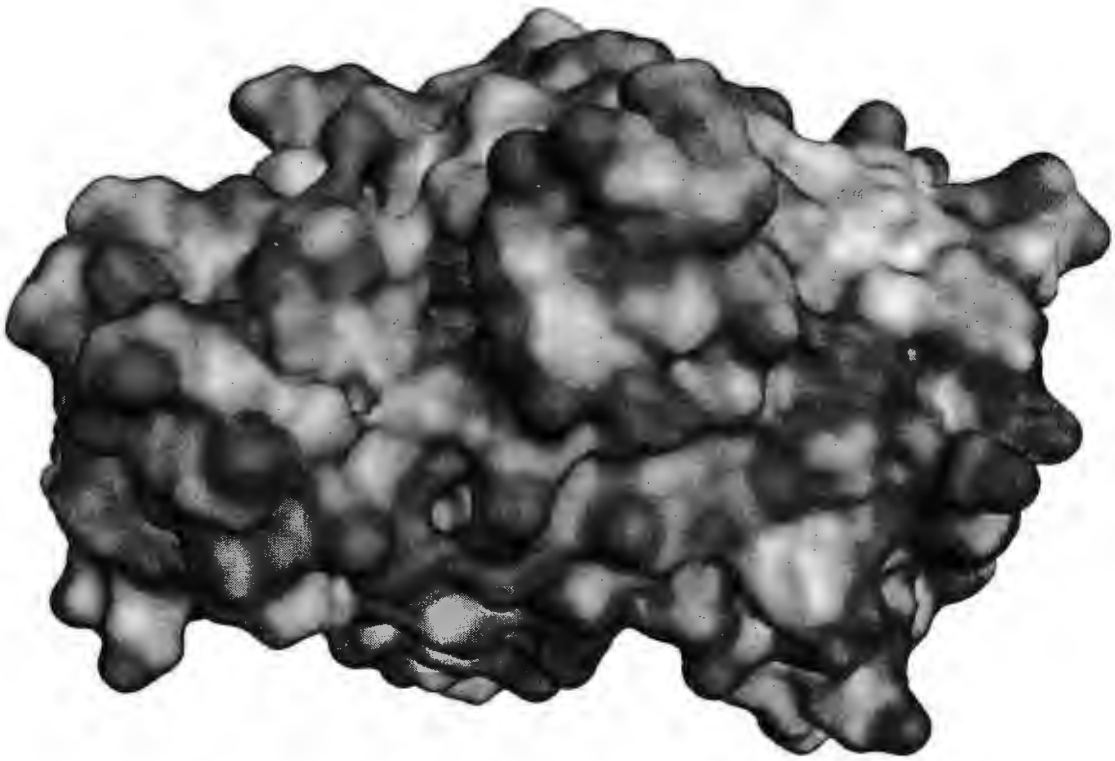


Figure 4.3: The 3D representation of the best fitting of compound NAS91 in the active site of FabZ. The blue, green and purple shows the surface of the FabZ while the NAS91 was shown by the ball and stick model.

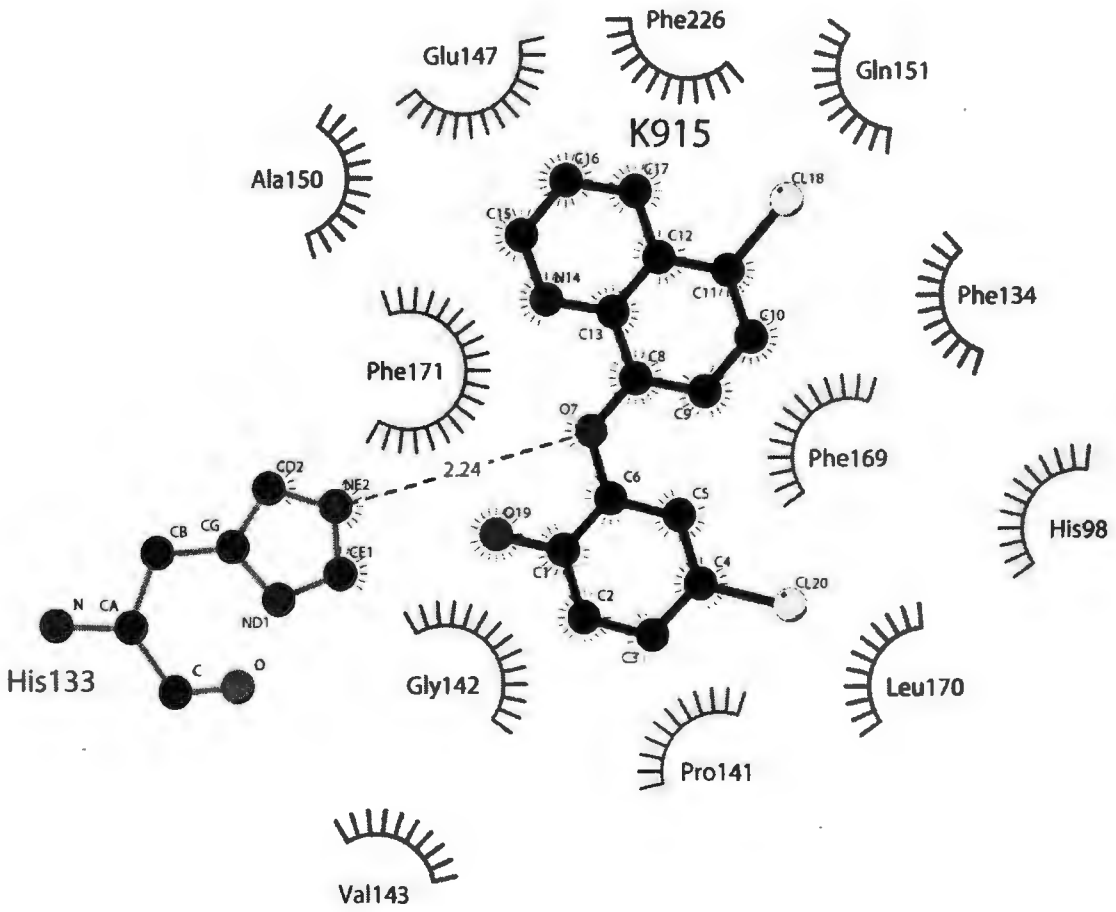
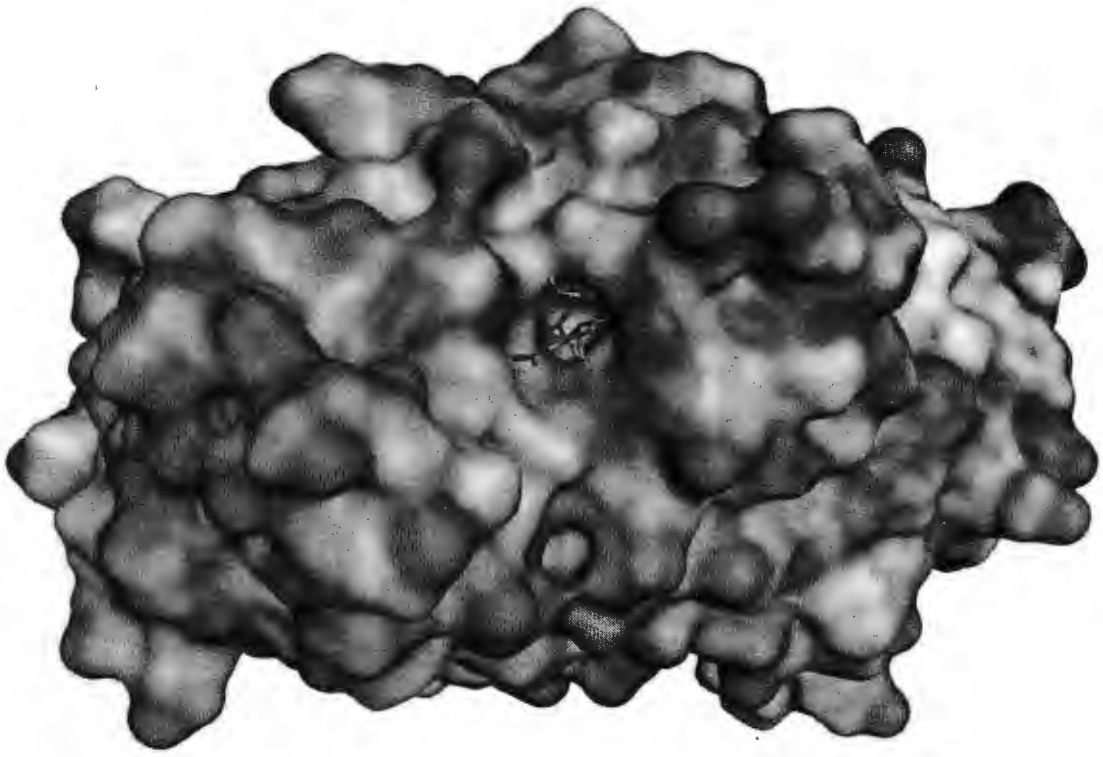


Figure 4.4: The 2D representation of the interaction of NAS91 in the active site of FabZ. The green dotted line shows the hydrogen bond while the spikes in red color shows the residues with hydrophobic interaction.

The chembridge compound with accession no **12407020** bind tightly in the active site of FabZ (Figure 5). The binding energy and binding affinity of the compound **12407020** were calculated as -13.128 kcal/mol and 10.723 pKi respectively. As compared to already reported inhibitor NAS91, the binding energy and binding affinity of the compound is very good. The compound **12407020** make numerous interaction with the active site residues. It make four strong hydrogen bonds with active site residues Phe171, Met140 Gln145 and His133. The distance of the hydrogen bond with Phe171 is 3.24 angstrom. The distance of hydrogen bond with Met140 is found to be 2.07 angstrom. The distance of the hydrogen bond with residues Gtln145 and His133 were found to be 3.43 and 2.96 angstrom respectively (Figure 6).

Figure 4.5: The 3D representation of the best fitting of compound **12407020** in the active site of FabZ. The blue, green and purple shows the surface of the FabZ while the **12407020** was shown



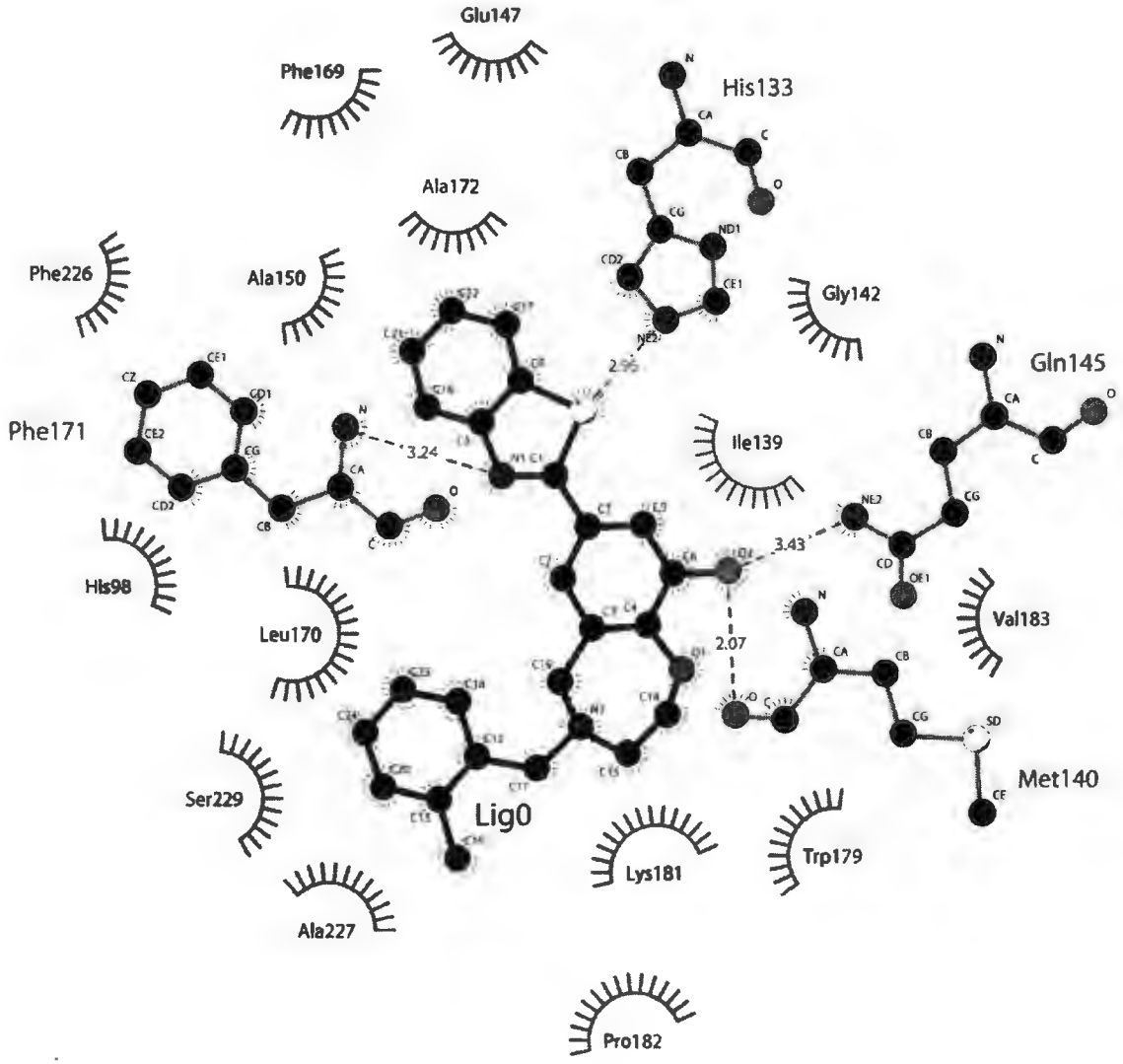


Figure 4.5The 3D representation of the best fitting of compound 12407020 by the ball and stick model.

Figure 4.6: The 2D representation of the interaction of **12407020** in the active site of FabZ. The green dotted line shows the hydrogen bond while the spikes in red color shows the residues with hydrophobic interaction.

The chembridge compound with accession no **10441542**, such molecule, with a molecular weight of 350.44 amu has a binding energy of -15.851KJ/Mol with the receptor. In the docked complex, among the interactions there is one hydrogen bond and 3 hydrophobic interactions between the molecule **10441542** and the protein. The conserved residues that are involved in hydrogen bonding include Gly142.

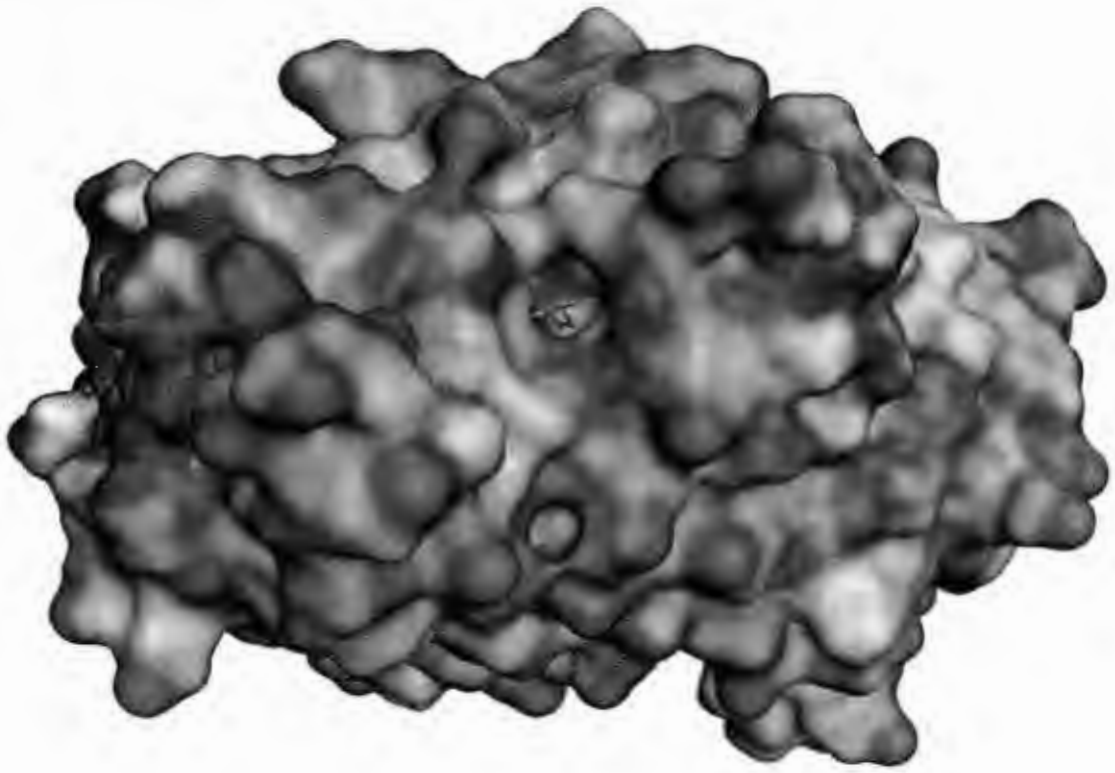


Figure 4.7: The 3D representation of the best fitting of compound 10441542 in the active site of FabZ. The blue, green and purple shows the surface of the FabZ while the 10441542 was shown

by the ball and stick model.

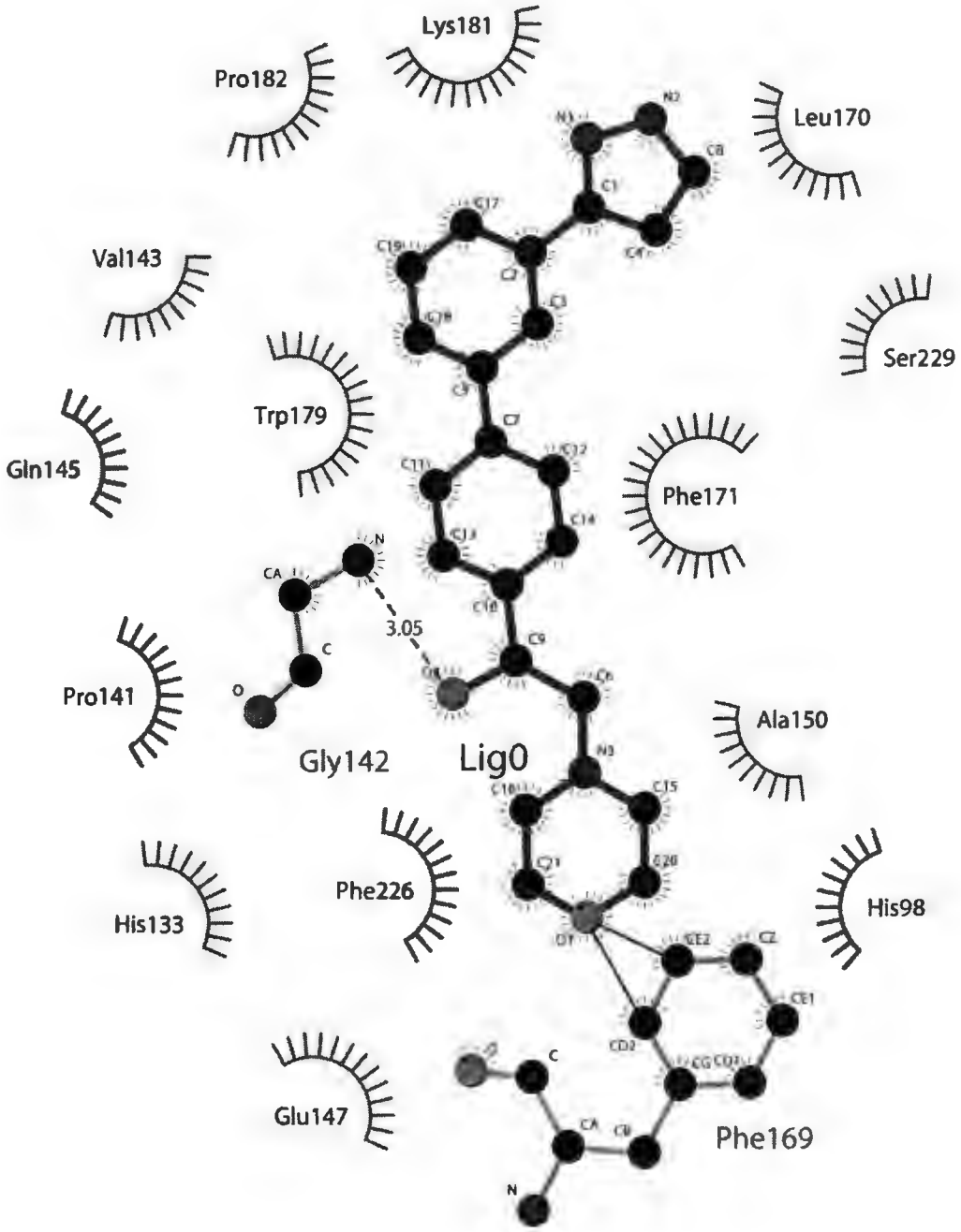


Figure 4.8: The 2D representation of the interaction of 10441542 in the active site of FabZ. The green dotted line shows the hydrogen bond while the spikes in red color shows the residues with hydrophobic interaction.

The chembridge compound with accession no 11235803, the molecule, with a molecular weight of 395.52 amu, has a binding energy of -13.497KJ/Mol with the receptor. In the docked complex, among the interactions there is 1 hydrogen bond and 5 hydrophobic interactions between the molecule 11235803 and the protein. The conserved residues that are involved in hydrogen bonding include Phe171 and Ser 23.

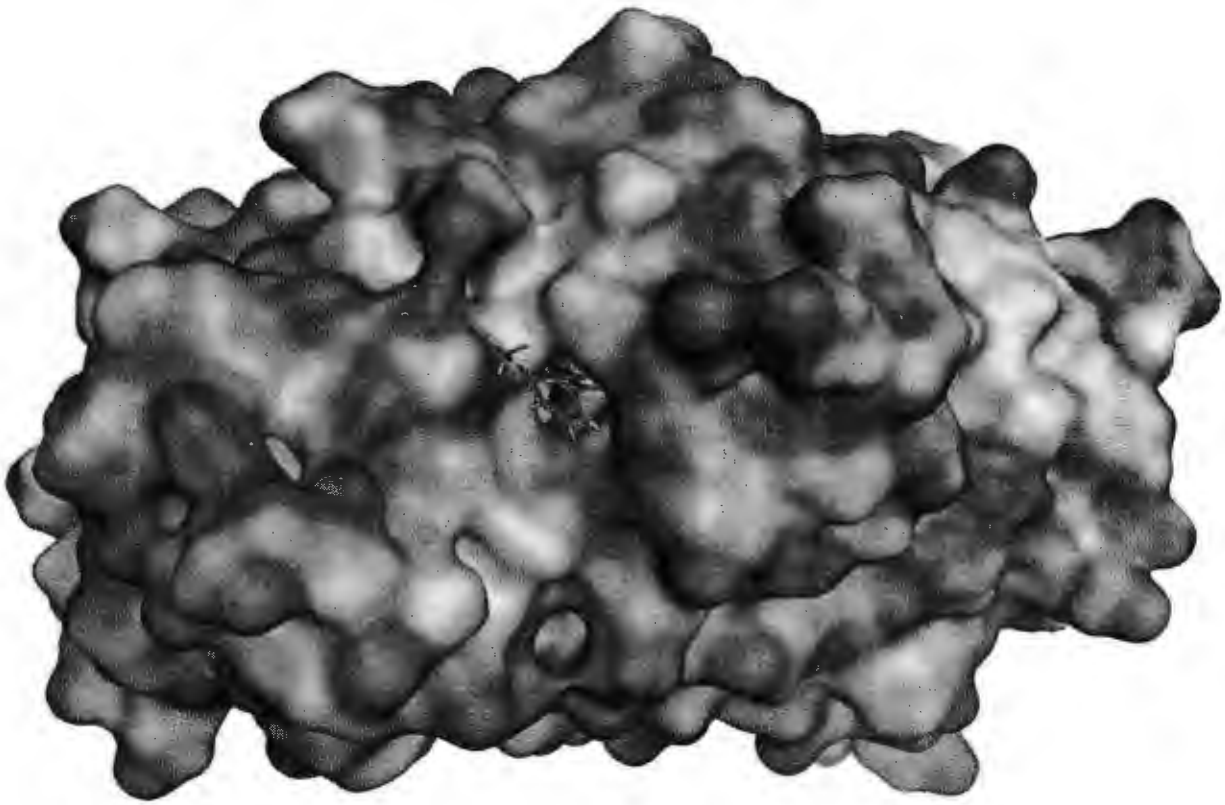


Figure 4.9: The 3D representation of the best fitting of compound 11235803 in the active site of

FabZ. The blue, green and purple shows the surface of the FabZ while the 11235803 was shown by the ball and stick model.

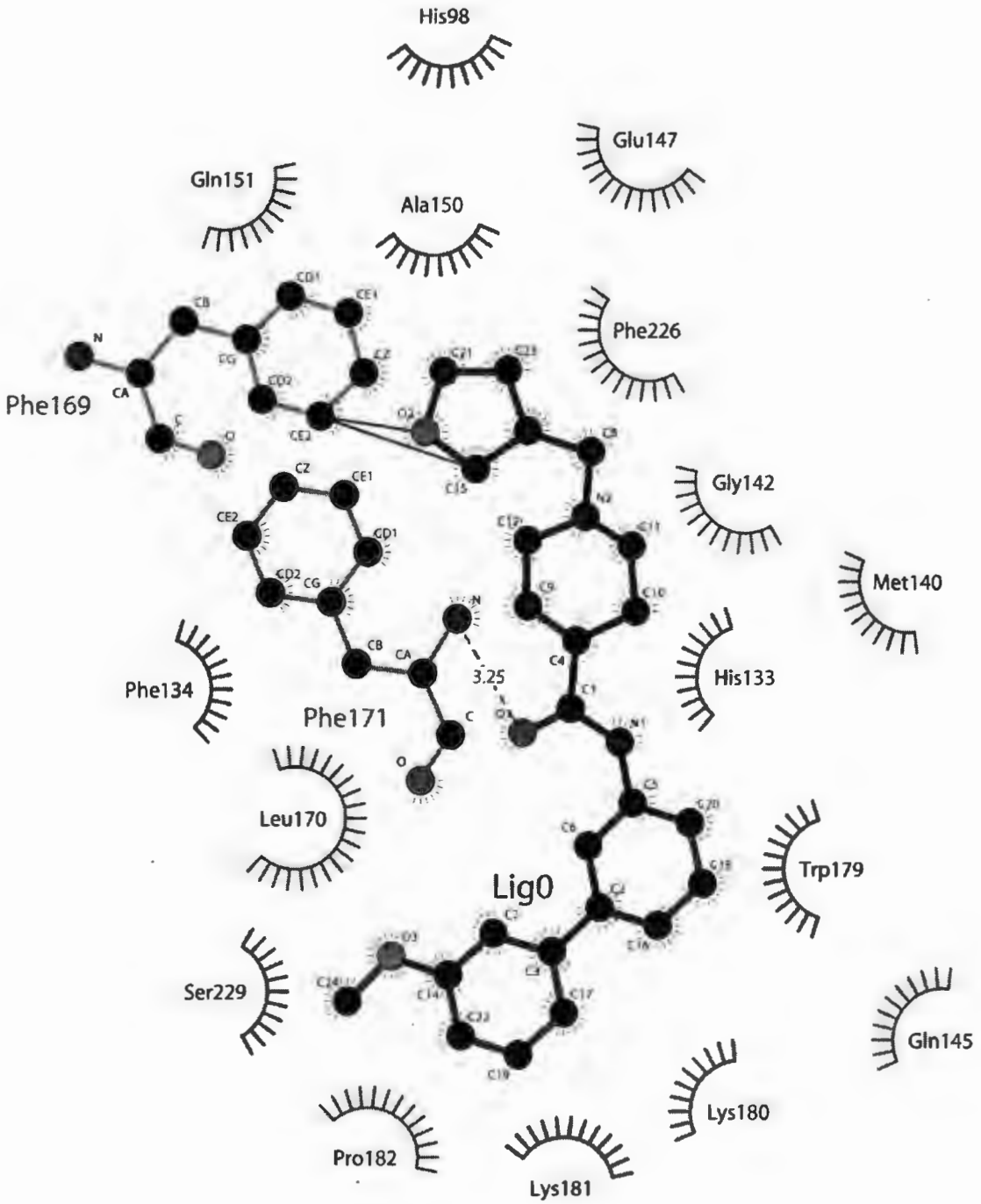


Figure 4.10: The 2D representation of the interaction of 11235803 in the active site of FabZ. The green dotted line shows the hydrogen bond while the spikes in red color shows the residues with hydrophobic interaction.

The chembridge compound with accession no **12083893** bind tightly in the active site of FabZ. The binding energy and binding affinity of the compound **12083893** were calculated as -14.401kcal/mol and 11.008pKi respectively. As compared to already reported inhibitors, the binding energy and binding affinity of the compound is very good. The compound **12083893** make numerous interaction with the active site residues. It make 2 strong hydrogen bonds with active site residues Lys181 and Gln145 and 6 hydrophobic interactions.

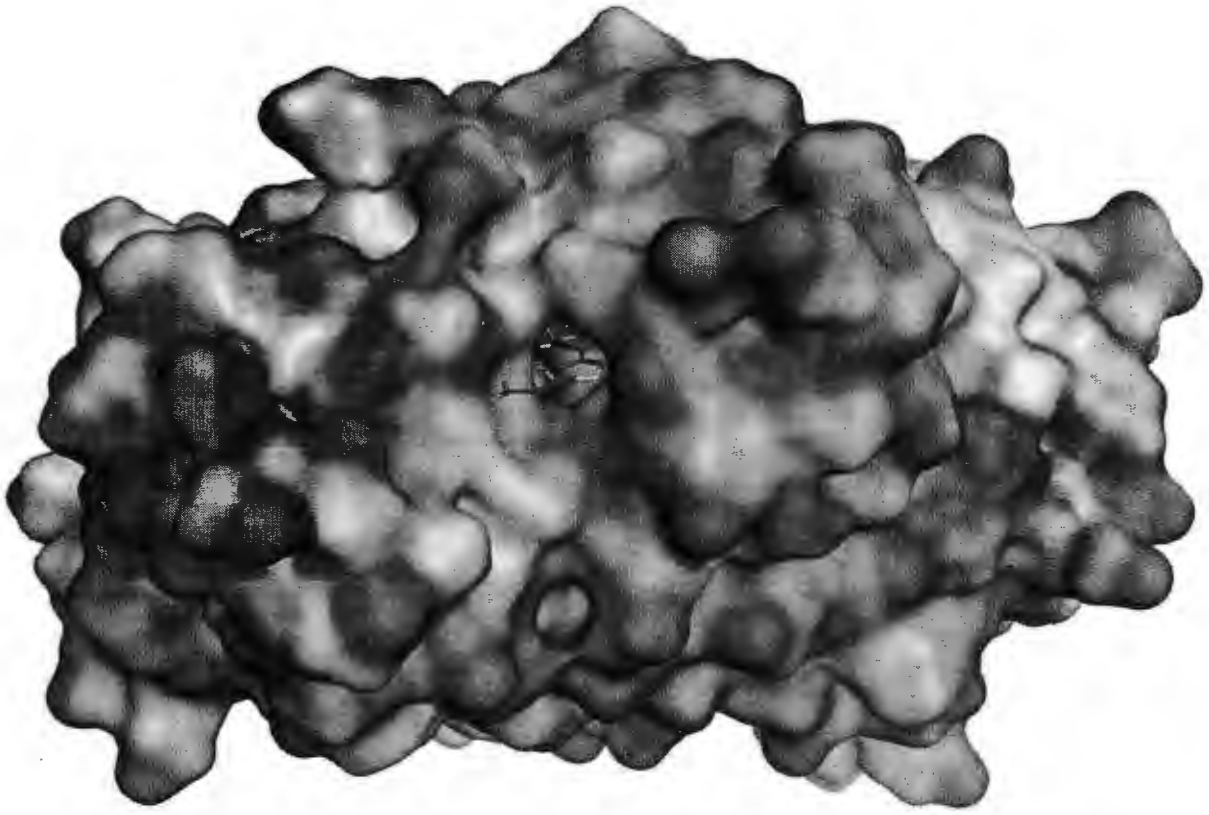


Figure 4.11: The 3D representation of the best fitting of compound **12083893** in the active site of FabZ. The blue, green and purple shows the surface of the FabZ while the **12083893** was shown by the ball and stick model.

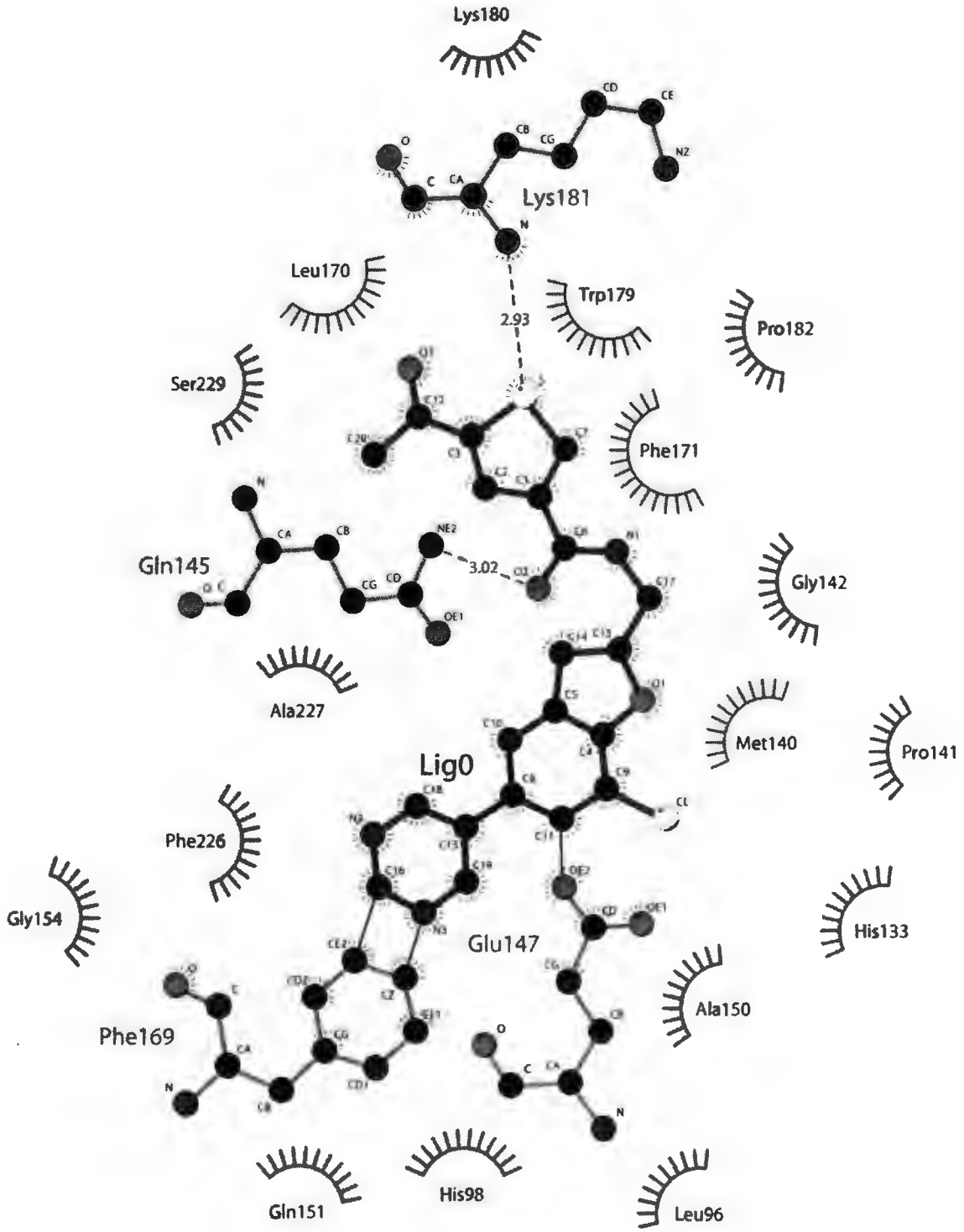


Figure 4.12: The 2D representation of the interaction of 11235803 in the active site of FabZ. The green dotted line shows the hydrogen bond while the spikes in red color shows the residues with hydrophobic interaction

The chembridge compound with accession no 12889810, the molecule, with a molecular weight of 395.568amu, has a binding energy of -11.576KJ/Mol with the receptor. In the docked complex, among the interactions there is no hydrogen bonds and 7 hydrophobic interactions between the molecule 12889810 and the protein. The residues of the active site such as His98, Phe171, Pro182, Gly142, Val143, Glu147, Ala150, Gln151, Phe226, Leu170, Phe171 and Phe226 were found to have hydrophobic interactions.

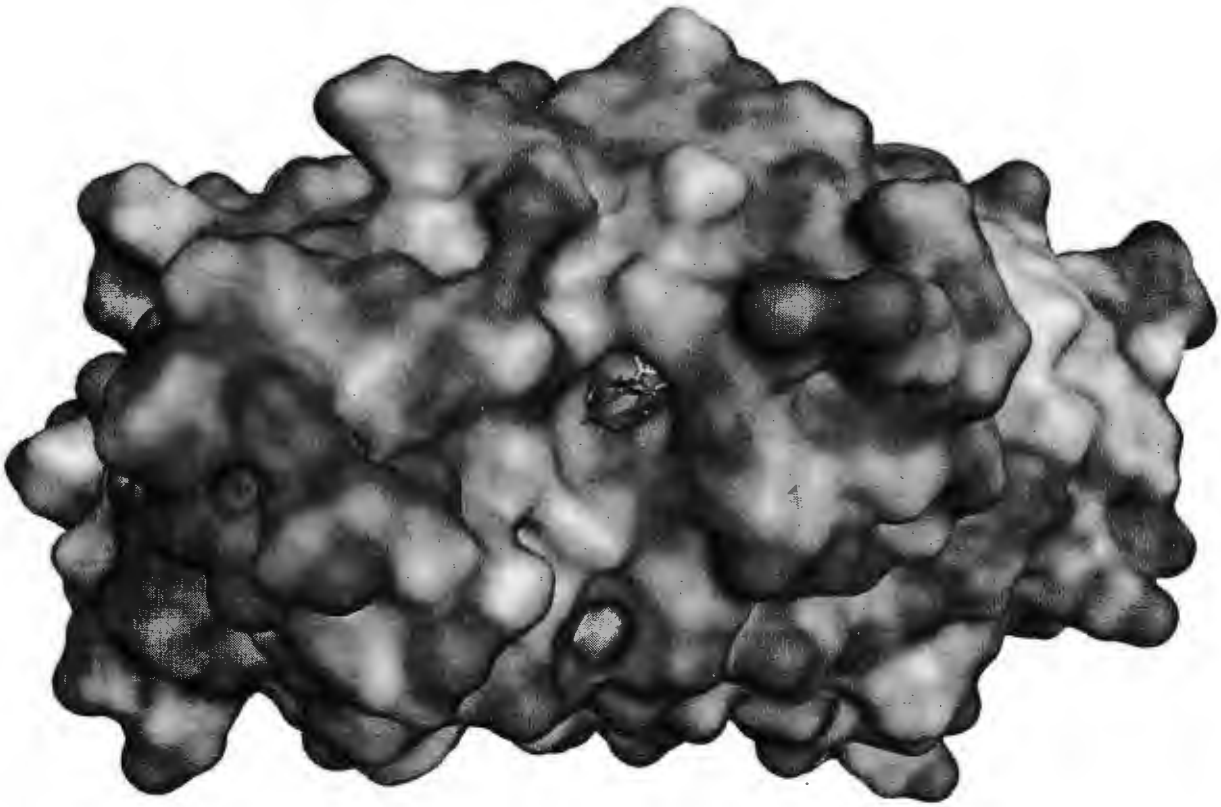


Figure 4.13: The 3D representation of the best fitting of compound 12889810 in the active site of FabZ. The blue, green and purple shows the surface of the FabZ while the 12889810 was shown by the ball and stick model.

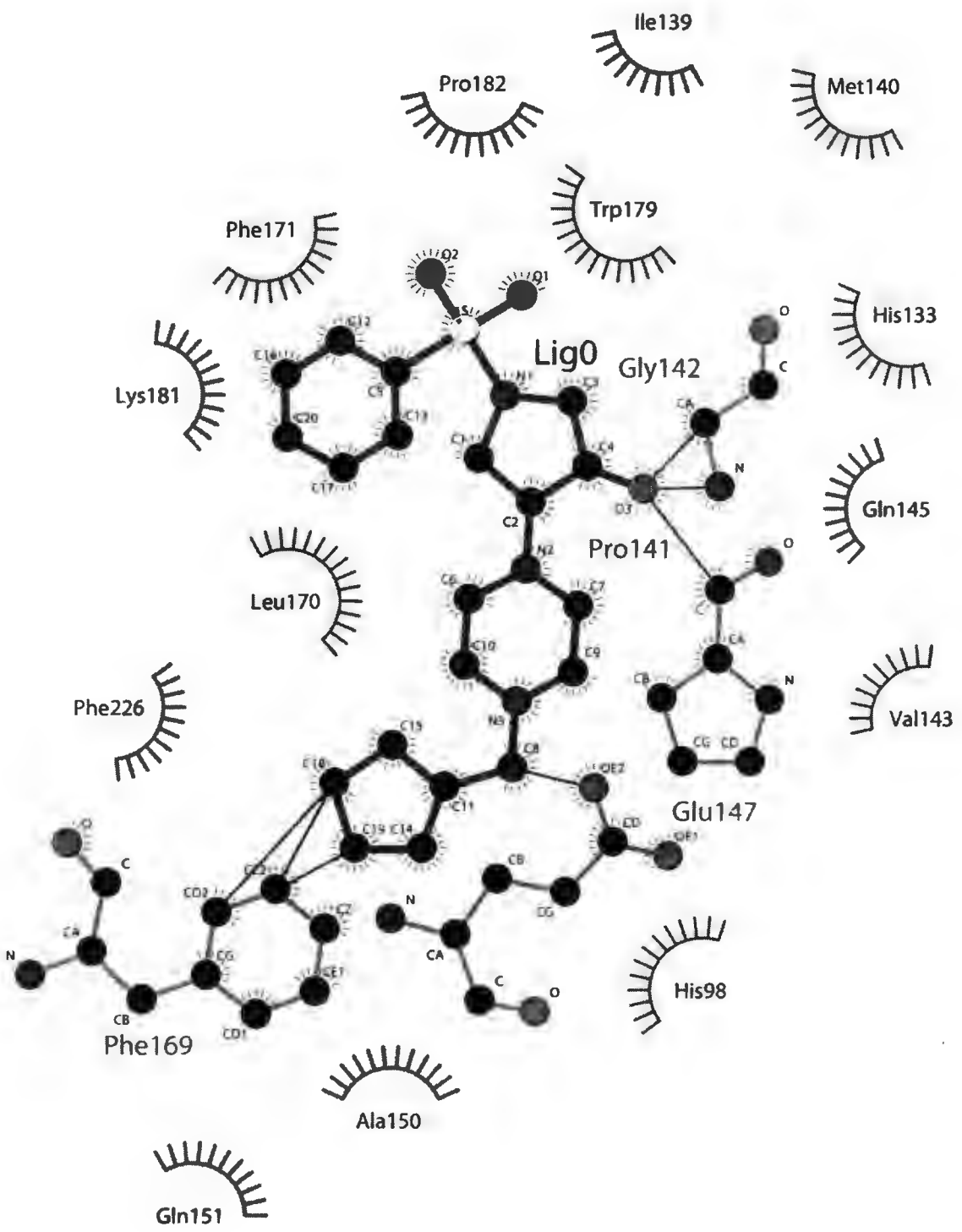


Figure 4.14: The 2D representation of the interaction of 12889810 in the active site of FabZ. The spikes in red color shows the residues with hydrophobic interacti

4.4 Molecular Dynamics simulations

The docking procedure was used and the position of each compound was found in the active site of FabZ. The docking results give only static interactions but in vivo the interaction process between ligand and target is dynamic in nature. Therefore, dynamic simulation was performed on each complex to check the stability of each compound in the active site of FabZ. The stability of each complex was checked in term of root mean square deviation (RMSD). Figure 4.15 shows the RMSD graph of each complex. As the RMSD value for all the complexes is below 2 Å, it means that FabZ suffered no significant structural changes during the 20 ns of MD simulation. The RMSD values increased up to 1.5 Å during the first 10 ns and then fluctuates around 1 Å for the rest of simulation. The RMSD graph shows that the all compounds are more stable.

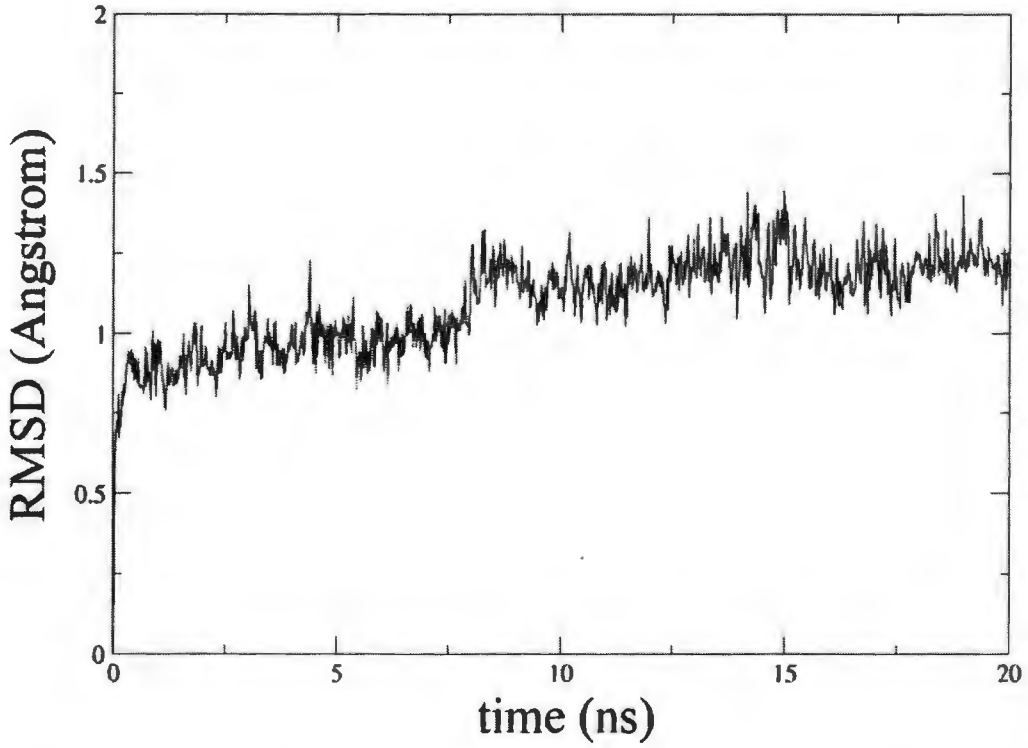


Figure 4.15: The RMSD graph of compound 10441542. The X-axis shows the length of MD simulation in nanosecond while the Y-Axis show the changes in structure by mean of root mean square deviation in angstrom

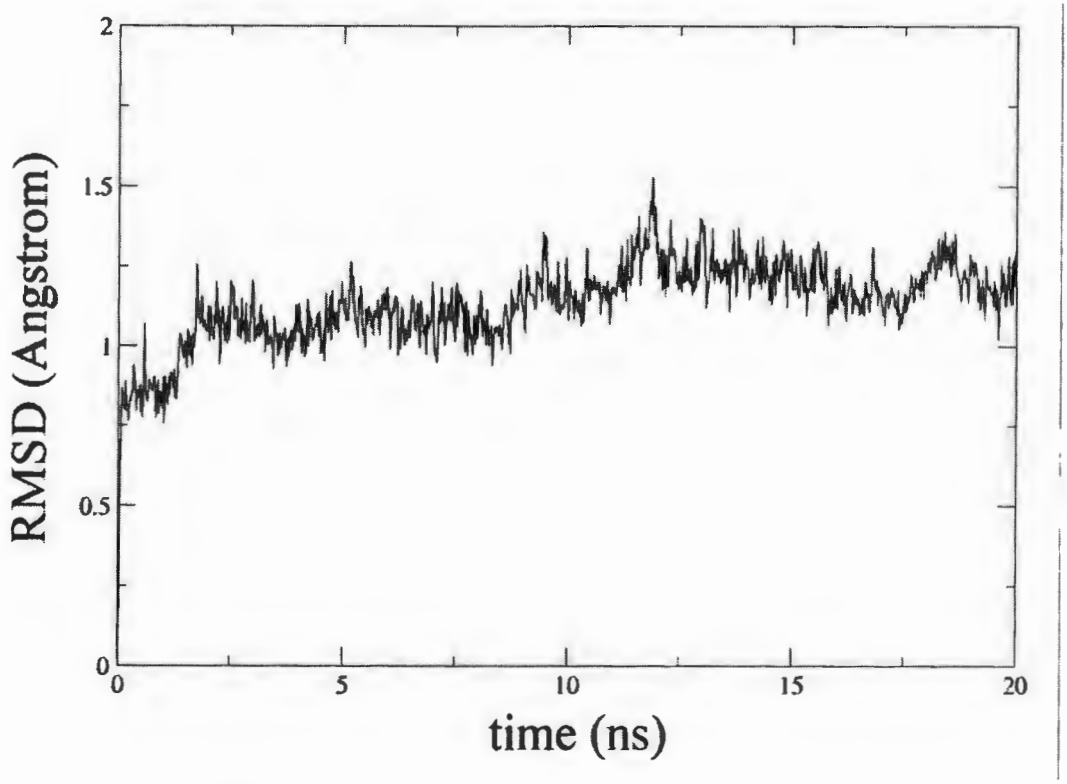


Figure 4.16: The RMSD graph of compound 11235803. The X-axis shows the length of MD simulation in nanosecond while the Y-Axis show the changes in structure by mean of root mean square deviation in angstrom.

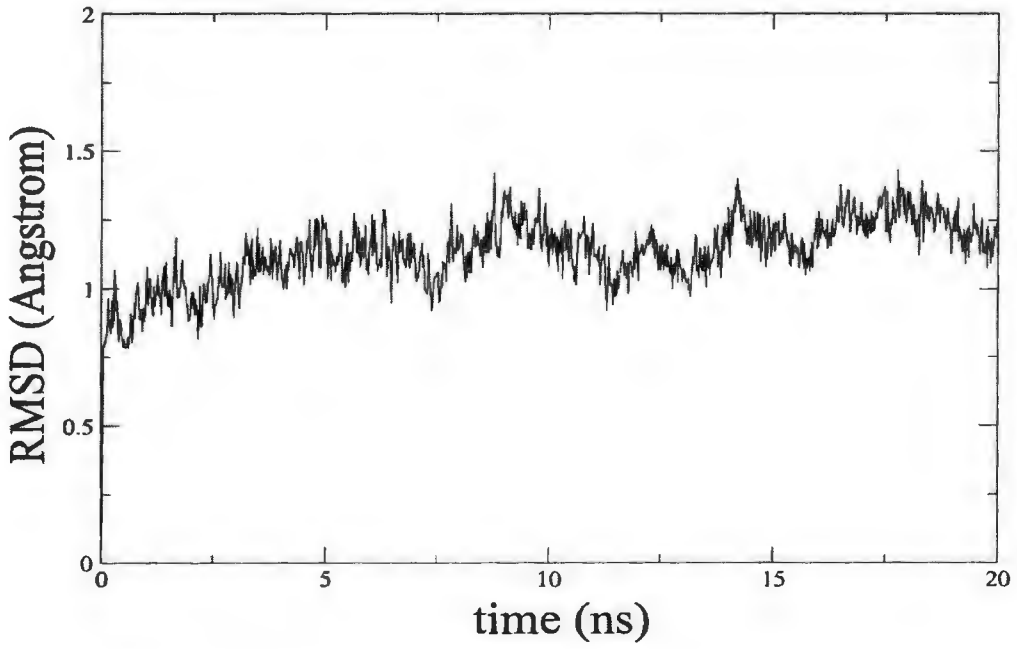


Figure 4.17: The RMSD graph of compound 12083893. The X-axis shows the length of MD simulation in nanosecond while the Y-Axis show the changes in structure by mean of root mean square deviation in angstrom

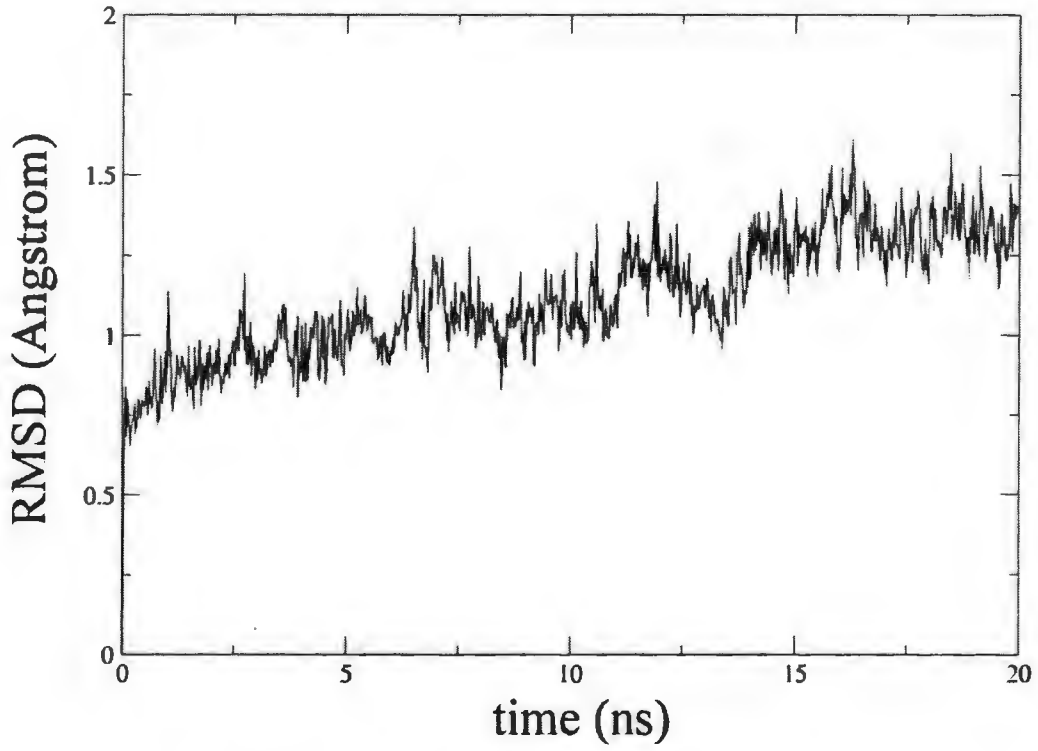


Figure 4.18: The RMSD graph of compound 12407020. The X-axis shows the length of MD simulation in nanosecond while the Y-Axis show the changes in structure by mean of root mean square deviation in angstrom

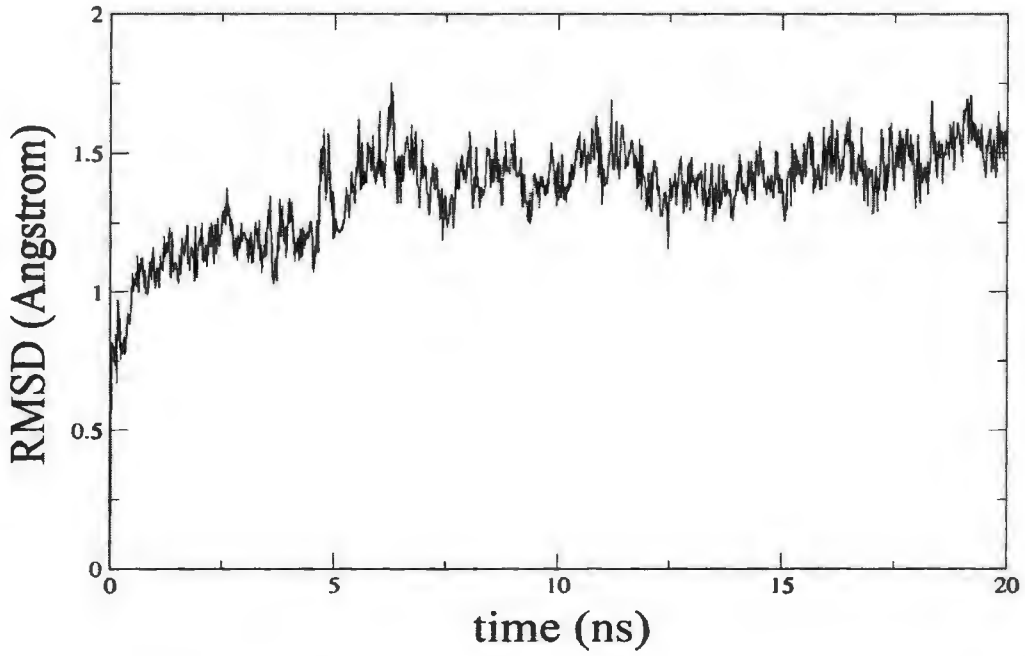


Figure 4.19: The RMSD graph of compound 12889810. The X-axis shows the length of MD simulation in nanosecond while the Y-Axis show the changes in structure by mean of root mean square deviation in angstrom

CHAPTER NO: 5

DISCUSSION

5. Discussion

Novel drug targets are needed in order to design new effective drugs against Malaria, it should be a molecule essential for pathogens and non-essential for human, therefore inhibition of essential proteins would kill the pathogen but have no effect on human. Chain elongation during fatty acid biosynthesis in *P. falciparum* is carried out by the β-hydroxyacyl- acyl carrier protein dehydratase (PfFabZ).

FabZ is a fundamental enzyme of fatty acid biosynthesis pathway which is absent in human and reported as the drug target molecule of malaria. The 3D structure of FabZ from Plasmodium falciparum was downloaded from PDB database (PDB ID: 3AZ9) and validated by computational tools, *in silico* docking studies were performed MOE and visualize using LIGPLOT v.4.5.3. LIGPLOT is a program for automatically plotting protein-ligand interactions. Overall validation results confirmed that the 3D structure was good, reported literature showing His98, Phe134, Pro141, Gly142, Val143, Glu147, Ala150, Gln151, Phe169, Leu170, Phe171 and Phe226 amino acids residues in the active site in the FabZ protein.

Our predictions with various active site prediction software are validating. Ligplot analysis was performed for FabZ protein against the database collected from ChemBridge database. Screened molecules were subjected to the docking with the FabZ protein. Some of the commercially available inhibitors like NAS91 and NAS91-10 taken as reference molecules for comparative analysis with the screened molecules.

Molecular docking result of the FabZ with the screened molecules and reference molecules were analyzed. The top 6 ranking natural compounds based on GLIDE scores are listed in (Table 3.1) The GLIDE scores and Xscores of these compounds have ranges of -15.851 to -9.260 KJ/Mol. The top ranking molecules are Top six screened inhibitors showed comparatively lesser binding energy as compared to the commercially available molecules.

Out of 6 screened molecules, ChemBridge NAS91, has an affinity score of 6.764 pKi and -9.260Kcal/mol. From its docked model, we find one hydrogen bond interactions and 2

hydrophobic contacts interactions between the ligand and the protein, Receptor-ligand interaction is shown that the active site after superposition of the ternary complexes, The green dotted line shows the hydrogen bond while the spikes in red color shows the residues with hydrophobic interaction.

In silico docking simulation analysis shows that the compound fits into the active pocket of the FabZ of the *Plasmodium falciparum*, suggesting that this may be the major cause of the inhibitory mechanism. The compounds (ChemBridge10441542), (ChemBridge11235803), (ChemBridge12083893), (ChemBridge12407020), (ChemBridge12889810) and (ChemBridge-NAS91) have a binding energy of (-15.851 KJ/Mol), (-13.497 KJ/Mol), (-14.401 KJ/Mol), (-13.128 KJ/Mol), (-11.576 KJ/Mol), (-9.260KJ/Mol) respectively. They show a good fit in the active site of the FabZ in the analysis using *in silico* docking simulation.Compare with the commercially available inhibitors like NAS91 taken as reference molecules for comparative analysis with the screened molecules, the data suggest inhibitory mechanism is due to the blocking of the active site at the FabZ of the *Plasmodium falciparum*.

CONCLUSION AND FUTURE WORK

The present study reports the three-dimensional crystal structure of PfFabZ at 2.1 Å resolution as well as inhibition data of NAS91 towards this enzyme, providing a framework for the understanding of the reaction mechanism of this type of enzyme. Moreover, the essential role of PfFabZ in fatty acid biosynthesis makes it an interesting target for drug discovery, and the current structure means a promising advance in the development of new anti-malarials, as it will serve as a model for structure-based drug development. The identification of potential lead compounds with inhibitory activity against PfFabZ using computational structure-based database screening approaches is in progress.

This study demonstrates that the identification of the above molecules as potential candidates for the drugs against β-hydroxyacyl- acyl carrier protein dehydratase (PfFabZ), Using structure aided virtual screening tools and databases. Molecules have been characterized with the binding energy. Given the good affinities of these compounds to the target proteins, they have good potential to be developed into to good drugs against malaria in the future.

CHAPTER NO: 6

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